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### Article

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# Immunoglobulin heavy chain expression shapes the B cell receptor repertoire in human B cell development

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Developing B cells must pass a series of checkpoints that are regulated by membrane-bound  $Ig\mu$  through the  $Ig\alpha$ - $Ig\beta$  signal transducers. To determine how  $Ig\mu$  expression affects B cell development and Ab selection in humans we analyzed Ig gene rearrangements in pro-B cells from two patients who are unable to produce  $Ig\mu$  proteins. We find that  $Ig\mu$  expression does not affect  $V_H$ ,  $D$ , or  $J_H$  segment usage and is not required for human  $Ig\kappa$  and  $Ig\lambda$  recombination or expression. However, the heavy and light chains found in pro-B cells differed from those in peripheral B cells in that they showed unusually long CDR3s. In addition, the  $Ig\kappa$  repertoire in  $Ig\mu$ -deficient pro-B cells was skewed to downstream  $J\kappa$ s and upstream  $V\kappa$ s, consistent with persistent secondary V(D)J rearrangements. Thus,  $Ig\mu$  expression is not required for secondary V(D)J recombination in pro-B cells. However, B cell receptor expression shapes the Ab repertoire in humans and is essential for selection against Ab's with long CDR3s.

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## Introduction

Expression of immunoglobulin heavy chains ( $Ig\mu$ ) in pro-B cells induces pre-B cell development by assembly of a pre-B cell receptor (pre-BCR), which is a complex of  $Ig\mu$ , surrogate light chains ( $\psi L$ ), and two signal-transducing proteins,  $Ig\alpha$  and  $Ig\beta$  (1–6). Pre-BCR expression induces proliferative expansion and downregulation of recombinase-activating genes ( $RAG1$  and  $RAG2$ ), thereby ensuring allelic exclusion while selecting clones of cells with productive  $VDJ_H$  rearrangements (7). Mutations in  $mIgu$ ,  $\psi L$ , or  $Ig\alpha/Ig\beta$  genes that disrupt pre-BCR assembly impede B cell development at the pro-B cell stage in mice and humans (8–15).

Pre-BCR assembly is dependent on interaction between  $V_H$  variable regions and  $\psi L$ , and in the mouse the  $IgH$  repertoire is selected in part on the basis of this interaction (16–18). It has been proposed that  $V_H$  domains that pair well with  $\psi L$  are positively selected at the pre-B cell stage, whereas  $V_H$  domains that pair poorly with  $\psi L$  are not. However, selection differs in mouse fetal and adult B cell development such that  $V_H$ s that are counterselected in the adult are prominent in the fetal repertoire (18).

In contrast to mice, there is no apparent difference in the  $V_H$  repertoire between pro-B, pre-B, or fetal B cells and adult mature B cells in the human (19–30). However, there is selection for  $IgH$  genes during human B cell development as determined by the length of the

third complementary determining region (CDR3). Pro- and pre-B cells from adult bone marrow have longer CDR3s than mature B cells (31–33).

Following successful  $IgH$  assembly and pre-BCR expression, V(D)J recombination is targeted to the light chain ( $IgL$ ) genes (34–38). Those cells that produce in-frame  $IgL$  chains test their newly synthesized Igs for self-reactivity. In the mouse, B cells that produce self-reactive receptors are either deleted or arrested in development and undergo receptor editing (39–45). In contrast, less is known about receptor selection and the role of the BCR in regulating B cell development in humans. Here we report on the role of the BCR in  $Ig$  repertoire selection in two patients with different mutations in the  $Ig\mu$  gene that impairs BCR assembly.

## Methods

**Patient samples and cell preparation.** Bone marrow samples were obtained from two  $Ig\mu$ -deficient patients with either a homozygous cytidine insertion in the  $Ig\mu$  gene ( $Ig\mu^{-/-}$ ) or with a homozygous deletion of the  $Ig\mu$  locus ( $Ig\mu^A$ ) (see Results) (13, 46, 47) (C. Schiff, unpublished observations). Samples were obtained when the  $Ig\mu^A$  patient was 2 years old, the  $Ig\mu^{-/-}$  patient was 4 years old, and her  $Ig\mu^{+/-}$  brother (control) was 9 months old. The parents gave informed consent for this study. Bone marrow mononuclear cells were isolated by Ficoll gradients and  $CD34^+CD19^+$  pro-B cells were sorted on a

FACSVantage after labeling with FITC anti-CD34 and phycoerythrin anti-CD19 mAb's (Beckman Coulter, Brea, California, USA).

**RNA and RT-PCR.** Total RNA was extracted from  $10^4$ – $10^5$  purified cells using TRIzol Reagent (Life Technologies Inc., Rockville, Maryland, USA). RNA was reverse transcribed with Superscript II (Life Technologies Inc.) according to the manufacturer's instructions. For RT-PCR reactions, cDNA was amplified for 25 (actin), 35 ( $V_H$ - $C_\mu$ ), or 38 ( $V_K$ - $C_K$ ) cycles of 30 seconds at  $94^\circ\text{C}$ , 30 seconds at  $60^\circ\text{C}$ , and 30 seconds at  $72^\circ\text{C}$ , or for 40 cycles ( $V_\lambda$ - $C_\lambda$ ) of 30 seconds at  $94^\circ\text{C}$ , 30 seconds at  $55^\circ\text{C}$ , and 30 seconds at  $72^\circ\text{C}$ , with a final 10-minute extension at  $72^\circ\text{C}$  using Hot Star Taq DNA polymerase (QIAGEN Inc., Valencia, California, USA) and the following primers:  $V_\lambda$  consensus sense, 5'GGG(G/A)TC(T/C)CTGA(C/T/G)CG(A/C/G)TTCTCTGG(C/G)TCC3';  $C_\lambda$  antisense, 5'CACAC(T/C)AGTGTGGCCTTGTGGCTTG3'.  $V_{H1}$ ,  $V_{H3}$ ,  $V_{H4}$ ,  $C_\mu$ ,  $V_K$  consensus and  $C_K$  primers were described previously (48, 49). RT-PCR products were analyzed on 2% agarose gels and visualized by adding 0.3 pmol of  $^{32}\text{P}$ dATP to the PCR reaction.

**Cloning and sequencing.** PCR products were gel-purified (Qiaquick; QIAGEN Inc.) and cloned into TA vectors (Invitrogen, Carlsbad, California, USA). Double-stranded DNA sequences were obtained using antisense  $C_\mu$ ,  $C_K$ , or  $C_\lambda$  primers and Dye Terminator Cycle Sequencing (Applied Biosystems, Foster City, California, USA). Sequences were analyzed by comparison with Ig basic alignment search tool (BLAST). IgH CDR3 length was determined by counting amino acid residues between positions 94 and 102 (conserved tryptophan in all  $J_H$  segments) and D segments were identified following the criteria of Corbett et al. (50). Igk

and Ig $\lambda$  CDR3 length included amino acids between conserved cystein 88 and the phenylalanine residue embedded in  $J_K$  or  $J_\lambda$  (51). Nontemplate (N) nucleotides (52) found at  $V_K$ - $J_K$  or  $V_\lambda$ - $J_\lambda$  junctions were counted while template-dependent palindromic (P) nucleotides (53) were excluded. Differences in gene distribution were analyzed with  $\chi^2$  tests (Cochran-Mantel-Haenszel test) adjusted by the Bonferroni method for multiple testing, and they were considered significant when  $P$  values were less than or equal to 0.05.

## Results

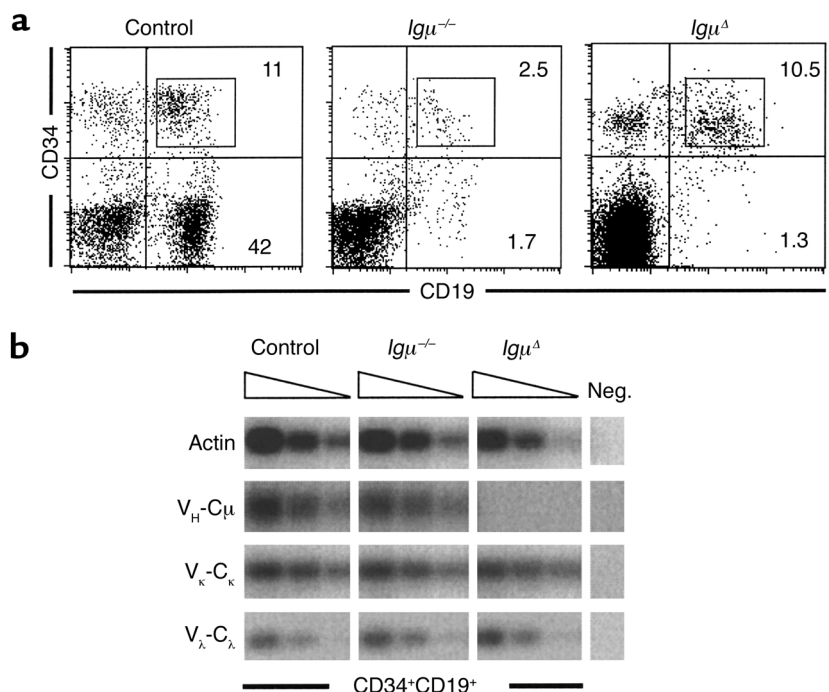
### *IgH and IgL transcription is independent of Ig $\mu$ expression.*

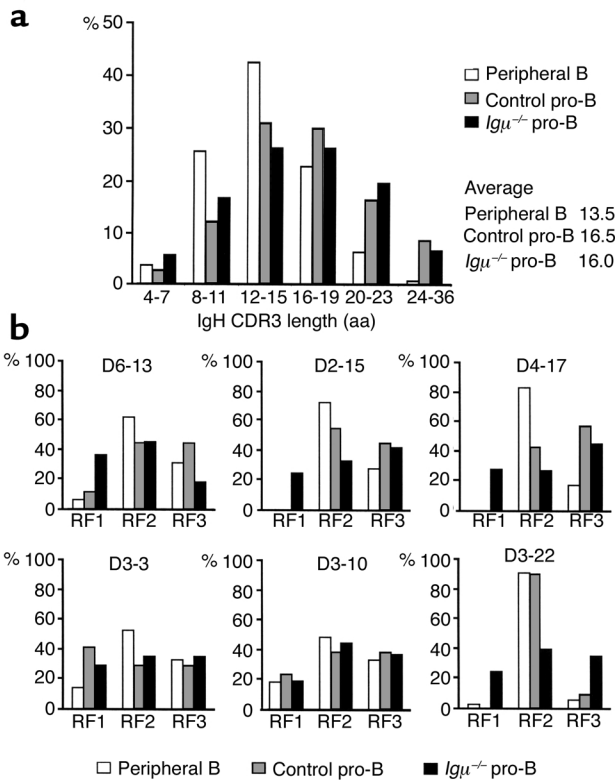
Two patients with agammaglobulinemia and IgH mutations were studied.  $Ig\mu^{-/-}$  has a cytidine insertion in the CH1 exon of the  $Ig\mu$  gene that leads to a frameshift and the inability to produce  $Ig\mu$  products (13, 46, 47).  $Ig\mu^\Delta$  has a deletion in the Ig locus from 3' of the diversity (D) region to  $Ig\gamma 2$ , with all junction (J) segments and  $Ig\mu$ ,  $Ig\delta$ ,  $Ig\gamma 3$ , and  $Ig\gamma 1$  genes missing (C. Schiff, unpublished observations).

Flow cytometric analysis of bone marrow from  $Ig\mu^{-/-}$  and  $Ig\mu^\Delta$  patients revealed that in both cases B cell differentiation was arrested at the  $CD34^+CD19^+$  pro-B cell stage (Figure 1a) (12, 13, 46, 47). To characterize Ig expression in  $Ig\mu$ -deficient pro-B cells, transcripts for heavy and light chain genes were amplified by semiquantitative RT-PCR from sorted  $CD34^+CD19^+$  pro-B cells from  $Ig\mu^{-/-}$ ,  $Ig\mu^\Delta$ , and a control sibling (Figure 1). As expected,  $V_H$ - $C_\mu$  mRNA was missing in  $Ig\mu^\Delta$  pro-B cells where the entire  $Ig\mu$  locus was deleted (Figure 1b). In contrast,  $Ig\mu^{-/-}$  and control pro-B cells showed similar levels of  $V_H$ - $C_\mu$  transcripts revealing that the absence of  $Ig\mu$  protein does not affect the  $Ig\mu$  gene expression in humans (Figure 1b).

**Figure 1**

Immunoglobulin rearrangements in human pro-B cells. (a) B cell precursors in sibling control (left),  $Ig\mu^{-/-}$  (middle), and  $Ig\mu^\Delta$  (right) bone marrow.  $CD34^+CD19^+$  pro-B cells from control and both  $Ig\mu$ -deficient patients were sorted as gated. (b) Heavy and light chain Ig gene expression in human pro-B cells. RNA from FACS-sorted  $CD34^+CD19^+$  pro-B cells from both  $Ig\mu$ -deficient patients and control was analyzed by semiquantitative RT-PCR using 5' consensus  $V_H$ ,  $V_K$ , or  $V_\lambda$  and 3'  $C_\mu$ ,  $C_K$ , or  $C_\lambda$  primers, respectively, and visualized by  $^{32}\text{P}$ dATP incorporation. "Neg." denotes a negative control without cDNA for RT-PCR reactions. Actin RT-PCR was used as mRNA loading control. Serial fivefold dilutions of cDNA are shown.





**Figure 2**

IgH CDR3 characteristics in pro-B cells. (a)  $V_HDJ_H$  CDR3 length in 350 peripheral B cell (white bars), 117 in-frame control pro-B (gray bars), and 197  $Ig\mu^{-/-}$  (black bars) individual sequences. CDR3 length in amino acids (aa) is indicated below. The average CDR3 length for peripheral B, control pro-B, and  $Ig\mu^{-/-}$  pro-B cells was 13.5, 16.5, and 16.0 amino acids, respectively. (b) D reading frame usage in IgH CDR3s from peripheral B, control, and  $Ig\mu^{-/-}$  pro-B cells. The three RF uses reported by Corbett et al. (50) for some commonly used D gene segments are represented. D3-3 and D3-10 encode no intra-genic stop codons whereas D6-13, D2-15, D4-17, and D3-22 sequences using RF1 display stop codons.

Thus, the pre-BCR is not essential for  $V_H$  selection, and intrinsic genetic factors are responsible for specific  $V_H$ , D, and  $J_H$  gene usage in human B cells.

**IgH CDR3 selection by  $Ig\mu$ .** To determine whether  $Ig\mu$  expression influences CDR3 selection, CDR3 length and amino acid composition were analyzed in  $Ig\mu^{-/-}$  pro-B cells and compared with control pro-B and peripheral B cells. About two-thirds of IgH genes were out of frame in  $Ig\mu^{-/-}$  pro-B cells, confirming the absence of  $Ig\mu$ -mediated selection in these cells (Table 1) (20). In contrast, two-thirds of IgH CDR3s were found to be in-frame in control  $CD34^+CD19^+$  precursor cells, suggesting early  $Ig\mu$ -mediated positive selection of a subpopulation of  $CD34^+CD19^+$  cells that express  $Ig\mu$  (Table 1). We compared IgH genes expressed by  $Ig\mu^{-/-}$  pro-B cells to the in-frame IgH genes expressed by control  $CD34^+CD19^+$  cells and found an average CDR3 length of 16.0 and 16.5 amino acids, respectively, whereas peripheral B cells showed an average CDR3 length of 13.5 amino acids (Figure 2a). D-D fusions that increase the length of CDR3 were found in about 2% (4 of 198) of the IgH sequences from  $Ig\mu^{-/-}$  pro-B cells and in 2.6% (3 of 117) of those from control  $CD34^+CD19^+$  cells, but were absent in peripheral B cells (49, 50) (data not shown). We conclude that IgH CDR3 length is not selected in early B cell precursors and that long CDR3s and D-D fusions are counterselcted during late stages of B cell development.

D segments can be used in three different reading frames, but in humans, RF1 tends to encode stop codons, RF2 usually encodes glycine residues, and hydrophilic amino acids and RF3 is biased to encode hydrophobic sequences (50). D segments in RF1 are

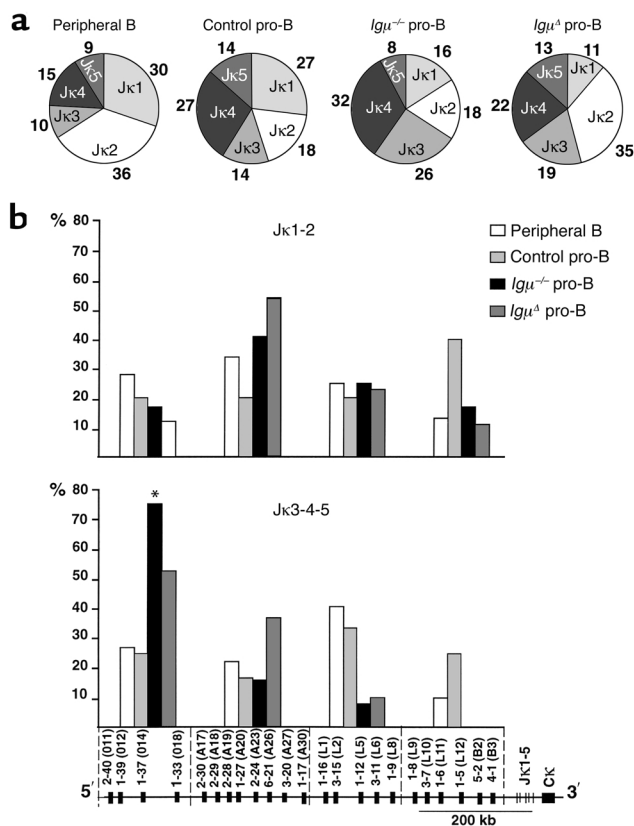
Light chain gene transcripts were found at similar levels in sorted control or  $Ig\mu$ -deficient pro-B cells (Figure 1b), but they were not amplified from  $Ig\mu^{-/-}$  total bone marrow cells that contain few pro-B cells (46, 47). We conclude that light chain genes can be recombined and expressed in the absence of  $Ig\mu$  in human pro-B cells.

**$Ig\mu$ -independent  $V_H$ , D, and  $J_H$  gene segment usage.** To determine whether  $Ig\mu$  expression is required for  $V_H$ , D, or  $J_H$  segment selection, IgH genes from the three major  $V_H$  families,  $V_H1$ ,  $V_H3$ , and  $V_H4$ , were cloned and sequenced.  $V_H$ , D, and  $J_H$  repertoire analysis revealed no statistically significant differences between  $Ig\mu^{-/-}$  pro-B cells, control pro-B cells, and peripheral B cells (19, 20, 26–30) (see supplemental data 1, [www.jci.org/cgi/content/full/108/06/879/DC1](http://www.jci.org/cgi/content/full/108/06/879/DC1)). Of 27 D genes in humans, only the D2-2 gene segment was over-represented in  $Ig\mu^{-/-}$  and control pro-B cells ( $P = 0.02$ ) (31, 50) (see supplemental data 2, [www.jci.org/cgi/content/full/108/06/879/DC2](http://www.jci.org/cgi/content/full/108/06/879/DC2)).

**Table 1**  
CDR3 frame analysis in  $CD34^+CD19^+$  pro-B cells

CDR3	Control			$Ig\mu^{-/-}$			$Ig\mu^A$	
	Frame			Frame			Frame	
	Out	In	Stop codon <sup>A</sup>	Out	In	Stop codon <sup>A</sup>	Out	In
H	69 (33%)	141 (67%)	11 (7.8%)	143 (73%)	54 (27%)	24 (44%)	–	–
$\kappa$	10 (45%)	12 (55%)		21 (55%)	17 (45%)		25 (68%)	12 (32%)
$\lambda$	11 (55%)	9 (45%)		6 (60%)	4 (40%)		15 (54%)	13 (46%)

<sup>A</sup>Stop codon frequency is calculated among in-frame CDR3s.



**Figure 3**

Igk light chain repertoire in pro-B cells. (a)  $J_{\kappa}$  usage in 108 peripheral B, 22 control pro-B, 38  $Ig\mu^{-/-}$ , and 37  $Ig\mu^{\Delta}$  pro-B  $V_{\kappa}DJ_{\kappa}$  individual sequences. Percentages of  $J_{\kappa}$  usage are indicated. (b)  $V_{\kappa}$  usage in upstream  $J_{\kappa}1$  and  $J_{\kappa}2$  ( $J_{\kappa}1-2$ ; top) and downstream  $J_{\kappa}3$ ,  $J_{\kappa}4$ , and  $J_{\kappa}5$  ( $J_{\kappa}3-4-5$ ; bottom) rearrangements of peripheral B cells (white bars), control pro-B (light gray bars),  $Ig\mu^{-/-}$  (black bars), and  $Ig\mu^{\Delta}$  (dark gray bars) pro-B cells. The  $V_{\kappa}$  genes are subdivided in four groups on the locus (84). The percentages of each  $V_{\kappa}$  group are indicated on the y axis. \*Statistically significant difference ( $P < 0.001$ ).

*Ongoing Igκ recombination in Igu-deficient pro-B cells.*

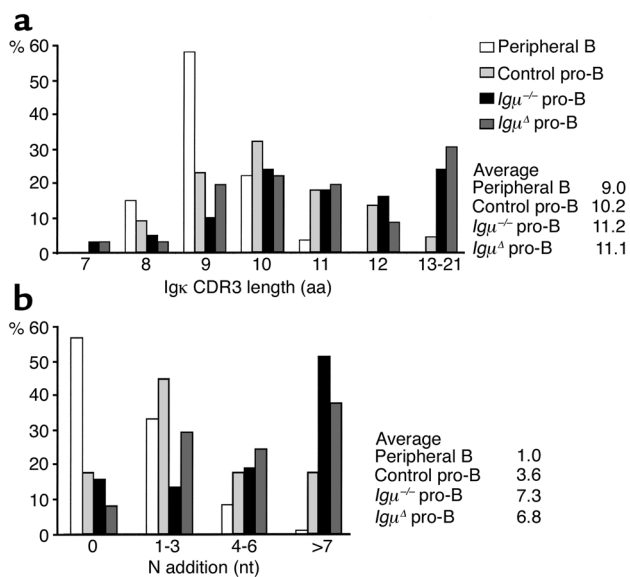
To characterize light chain gene expression in human pro-B cells, we amplified and sequenced Igκ mRNAs from  $Ig\mu^{-/-}$  and  $Ig\mu^{\Delta}$  pro-B cells and compared them with those of control pro-B and peripheral B cells (49). We found that Igκ mRNAs expressed in  $Ig\mu^{-/-}$  and  $Ig\mu^{\Delta}$  pro-B cells showed decreased  $J_{\kappa}1$  and increased downstream  $J_{\kappa}3$  usage when compared with normal B cell controls (Figure 3a). In addition,  $J_{\kappa}3-4-5$  segments from  $Ig\mu^{-/-}$  and  $Ig\mu^{\Delta}$  pro-B cells were preferentially combined with upstream  $V_{\kappa}\kappa$  ( $P = 0.005$ , Figure 3b) whereas there was no such bias in the  $J_{\kappa}3-4-5$  Igκ mRNAs from normal pro-B cell and peripheral B cell controls. In contrast, there was no bias in  $V_{\kappa}$  gene usage for Igκ genes using  $J_{\kappa}1-2$  segments in patients and controls (Figure 3b). We conclude that in the absence of  $Ig\mu$  there is a shift in the Igκ repertoire to downstream  $J_{\kappa}\kappa$  and upstream  $V_{\kappa}\kappa$  consistent with secondary Igκ rearrangement in pro-B cells.

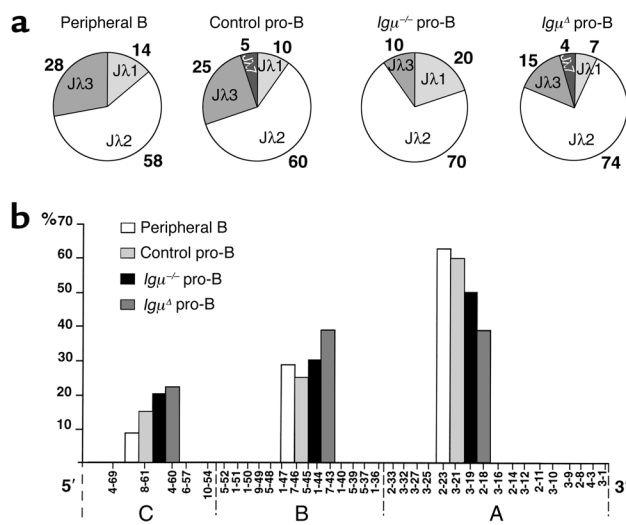
Igκ CDR3 analysis revealed that the ratio of productive to nonproductive  $V_{\kappa}\kappa$  joints in human pro-B cells was similar to that reported for  $mIgu$ -deficient mouse ( $\mu$ MT) pro-B cells (54). Igκ CDR3 length was increased in all pro-B cell samples when compared with peripheral B cells (Table 1 and Figure 4a). We found that Igκ CDR3s from control pro-B cells and from the patients had an average of 10.2, 11.2, or 11.1 amino acids where-

normally under-represented in peripheral B cells and were counterselected in control  $CD34^{+}CD19^{+}$  cells (Figure 2b, top and bottom row, and Table 1). In contrast, there was neither RF selection nor stop codon counterselection in  $Ig\mu^{-/-}$  pro-B cells (Figure 2b, top and bottom row, and Table 1).  $D3-3$  or  $D3-10$  genes that do not contain intragenic stop codons in RF1 were used in all three RFs in  $Ig\mu^{-/-}$ , control  $CD34^{+}CD19^{+}$ , or peripheral B cells (Figure 2b, middle row). In addition, hydrophilic (RF2) and hydrophobic (RF3)  $D$ s were used equally in  $Ig\mu^{-/-}$  and control  $CD34^{+}CD19^{+}$  cells whereas RF2 was favored in peripheral B cells (Figure 2b) (31, 50). However,  $D3-22$  RF usage was already selected in control  $CD34^{+}CD19^{+}$  cells since RF3 was clearly counterselected (and/or RF2 positively selected) when functional  $Ig\mu$  chains were generated (Figure 2b, bottom row). Thus, IgH CDR3s containing stop codons are counterselected in early B cell precursors whereas CDR3s with hydrophilic or hydrophobic RF are not.

**Figure 4**

Igκ CDR3 characteristics in pro-B cells. (a) Igκ CDR3 length in amino acids and (b) N nucleotide (nt) addition in peripheral B cells (white bars), control pro-B (light gray bars),  $Ig\mu^{-/-}$  (black bars), and  $Ig\mu^{\Delta}$  (dark gray bars) pro-B cells. For determination of N nucleotide addition, P nucleotides were not included. The average CDR3 length and N nucleotide addition for peripheral B, control pro-B,  $Ig\mu^{-/-}$ , and  $Ig\mu^{\Delta}$  pro-B cells was 9.0, 10.2, 11.2, and 11.1 amino acids and 1.0, 3.6, 7.3, and 6.8 nucleotides, respectively.





**Figure 5**

Igλ light chain repertoire in pro-B cells. (a) Jλ usage in 163 peripheral B, 20 control pro-B, 10 *Igμ*<sup>-/-</sup>, and 28 *Igμ*<sup>Δ</sup> pro-B VλJλ individual sequences. Percentages of Jλ usage are indicated. (b) Vλ usage in peripheral B cells (white bars), control pro-B (light gray bars), *Igμ*<sup>-/-</sup> (black bars), and *Igμ*<sup>Δ</sup> (dark gray bars) pro-B cells. The Vλ locus is shown clustered into three groups — A, B, and C — of Vλ genes (84). The percentages of each Vλ group are indicated on the y axis.

as Igκ CDR3 from peripheral B cells were only 9.0 amino acids long (Figure 4a). The increased CDR3 length in pro-B cells was due to terminal deoxynucleotidyl transferase-catalyzed (TdT-catalyzed) addition of template-independent (N) nucleotides and not to template-dependent P nucleotides (Figure 4b). On average, 3.6, 7.3, and 6.8 nucleotides were added by TdT to Igκ gene CDR3s in control, *Igμ*<sup>-/-</sup>, and *Igμ*<sup>Δ</sup> pro-B cells whereas only 1 N nucleotide was found in Igκ genes expressed by peripheral B cells (Figure 4b). We conclude that pro-B cells produce Igκ genes with unusually long CDR3s.

**Igλ repertoire of *Igμ*-deficient pro-B cells.** To determine whether Igλ genes expressed in *Igμ*-deficient pro-B cells displayed features similar to Igκ genes we analyzed the Igλ repertoire. Although the human Igλ locus should allow deletional replacement of VJλs by secondary recombination (55), we found no significant increases in either downstream Jλ or distal Vλ segment usage in *Igμ*<sup>-/-</sup> and *Igμ*<sup>Δ</sup> pro-B cells. In fact, there was a decrease in downstream Jλ.3 segment usage (56, 57) (Figure 5, a and b).

Igλ genes expressed in control, *Igμ*<sup>-/-</sup>, and *Igμ*<sup>Δ</sup> pro-B cells resembled Igκ genes in that they showed a similar ratio of in-frame and out-of-frame sequences and long CDR3s, resulting from addition of N nucleotides by TdT (Figure 6, a and b, and Table 1). The average number of N nucleotides in Igλ CDR3s from control, *Igμ*<sup>-/-</sup>, and *Igμ*<sup>Δ</sup> pro-B cells was 3.9, 5.0, and 6.5 as compared with 1.2 for mature B cells (Fig-

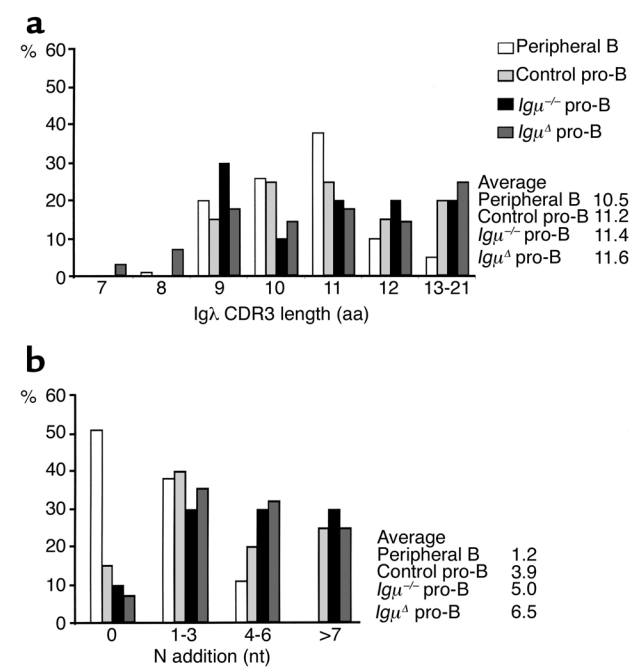
**Figure 6**

Igλ CDR3 characteristics in pro-B cells. (a) Igλ CDR3 length in amino acids and (b) N nucleotide addition in peripheral B cells (white bars), control pro-B (light gray bars), *Igμ*<sup>-/-</sup> (black bars), and *Igμ*<sup>Δ</sup> (dark gray bars) pro-B cells. The average CDR3 length and N nucleotide addition for peripheral B, control pro-B, *Igμ*<sup>-/-</sup>, and *Igμ*<sup>Δ</sup> pro-B cells was 10.5, 11.2, 11.4, and 11.6 amino acids and 1.2, 3.9, 5.0, and 6.5 nucleotides, respectively.

ure 6b). We conclude that the Igλ genes expressed by *Igμ*<sup>-/-</sup> and *Igμ*<sup>Δ</sup> pro-B cells differ from Igκ genes in that they show no signs of secondary recombination, but they resemble Igκ genes in that they display long CDR3s with extensive N addition.

## Discussion

The absence of *Igμ* in *Igμ*<sup>-/-</sup> and *Igμ*<sup>Δ</sup> patients provided an opportunity to study the role of *Igμ* in selecting the Ab repertoire in humans. We found no significant differences in the Ig heavy chain gene V<sub>H</sub>, D, or J<sub>H</sub> repertoire between *Igμ*-deficient pro-B cells, control pro-B cells, and normal peripheral B cells. These findings are in agreement with cell-sorting experiments in which normal pro-B, pre-B, and immature B cell repertoires were compared with that of peripheral B cells (19–21, 31). Thus, V<sub>H</sub>, D, and J<sub>H</sub> segment usage in humans is independent of *Igμ* expression and is likely to be a function of intrinsic genetic elements controlling V<sub>H</sub>, D, or J<sub>H</sub> gene accessibility and recombination. In contrast, IgH CDR3 length appears to be selected throughout B cell development starting with CD34<sup>+</sup>CD19<sup>+</sup>IgM<sup>-</sup> pre-B cells (33). Our analysis of *Igμ*<sup>-/-</sup> and control CD34<sup>+</sup>CD19<sup>+</sup> cells showed that *Igμ* expression is not involved in IgH CDR3 length and hydrophilic RF selection in the human at the pro-B cell stage. However, in-frame IgH genes without stop



codons were enriched in control CD34<sup>+</sup>CD19<sup>+</sup> B cell progenitors, suggesting that an efficient selection process driven by pre-BCRs operates in normal CD34<sup>+</sup>CD19<sup>+</sup> B cell precursors. These I $\mu$ -positive B cell precursors displaying surface pro-B cell markers are likely to be in transition to the pre-B cell stage and equivalent to the mouse C' early pre-B cell fraction of Hardy's classification (58). By analogy to I $\mu$  or I $\beta$  knockout mice, the C' early pre-B cell fraction is missing in I $\mu$ <sup>-/-</sup> CD34<sup>+</sup>CD19<sup>+</sup> precursor B cells and results in a decrease of in-frame I $\mu$  rearrangements when compared with normal CD34<sup>+</sup>CD19<sup>+</sup> precursor B cells (10, 59).

In the mouse,  $\Psi$ L has been implicated in IgH repertoire selection by virtue of pairing with some but not all V<sub>H</sub> domains (16, 18). Analysis of the V<sub>H</sub> repertoire in  $\lambda$ 5<sup>-/-</sup> mice showed that the normal repertoire shift seen between the pro-B and the pre-B cell stage was absent (16). However, differences in pairing efficiency between  $\Psi$ L and IgH are not likely to influence the selection against long or hydrophobic CDR3s in humans because these features are prevalent in control CD34<sup>+</sup>CD19<sup>+</sup> cells, in immature B cells that have passed  $\Psi$ L selection, and in B cells that express  $\Psi$ Ls in the periphery (31, 49). A more likely explanation for selection against long and hydrophobic IgH CDR3s is that these features are associated with self-reactivity and might also interfere directly with IgH and IgL pairing (31, 49, 60, 61). Immature B cells displaying such Ab's therefore would be silenced by deletion or receptor editing, or alternatively, would fail to be positively selected in the mature B cell compartment (39, 40, 42–44, 62–64).

In the mouse, two I $\mu$ -mediated mechanisms account for selection against self-reactive or poorly pairing Ab's during B cell development, receptor editing and deletion (39–44). Editing makes a major contribution to the Ab repertoire in mice: up to 25% of all Ab's are produced by editing, but the role of deletion in repertoire selection is not known (65). Our experiments suggest that I $\mu$ -mediated selection also makes a large contribution to shaping the human Ab repertoire. The selection against IgHs with long or hydrophobic CDR3s found in pro-B cells would require loss of at least 20–25% of all heavy chains.

In both mouse and human B cells, V(D)J recombination is generally ordered, starting with IgH rearrangement in pro-B cells followed by IgL rearrangement in pre-B cells (66, 67). However, analysis of mouse I $\mu$  mutants and normal pro-B cells showed that IgL genes can recombine before IgH in pro-B cells (54, 68–71). Our experiments show that human control and I $\mu$ -deficient pro-B cells are similar to their mouse counterparts in that they undergo I $\mu$  and I $\lambda$  gene rearrangements. These results are in agreement with the finding of rare IgL chain gene recombination in normal human pro-B cells and in Epstein-Barr virus-transformed fetal B cell precursors (67, 72). Thus, IgL rearrangement in the human is similar to the mouse in that it is not strictly dependent on I $\mu$  expression or pre-B cell development.

Up to 50% of human light chains in normal peripheral B cells show N nucleotide addition, but I $\mu$  or I $\lambda$  CDR3s are never as long as 11 or 13 amino acids, respectively (49, 73–76). I $\mu$  or I $\lambda$  genes found in control and I $\mu$ -deficient pro-B cells differ from those found in normal peripheral B cells in that they show extensive N nucleotide addition associated with long IgL CDR3s that can reach up to 21 amino acids. Thus, long IgL CDR3s are produced in early developing B cells but they appear to be incompatible with B cell development and are deleted from the mature peripheral B cell repertoire.

I $\mu$ -deficient pro-B cells express I $\mu$  genes that display a bias to 3' J $\mu$ s and 5' V $\mu$ s consistent with persistent V(D)J recombination. However, the bias to 3' J $\mu$ s was incomplete since there was no significant increase in J $\mu$ 5, the most downstream J $\mu$  segment. In addition, there was no bias to downstream J $\mu$ s despite a genomic configuration that allows secondary rearrangements (55, 77, 78). We speculate that in the absence of I $\mu$ , human pro-B cells undergo several rounds of recombination on I $\mu$  but do not survive long enough to allow extensive secondary recombination. The absence of secondary recombination on I $\lambda$  in I $\mu$ -deficient pro-B cells may result from a delayed recombination of this locus when compared with I $\mu$  (79, 80). Alternatively, secondary recombination may be less efficient for I $\lambda$  than I $\mu$ .

Secondary I $\mu$  recombination is prominent in I $\mu$ -deficient pro-B cells yet not in normal control pro-B cells. Secondary recombination in I $\mu$ -deficient pro-B cells therefore appears to be a default mechanism in the absence of I $\mu$  expression, and termination of secondary recombination in developing B cells requires BCR signaling. We speculate that the regulation of Ig light chain gene recombination during B cell development resembles that of T cell receptor- $\alpha$  (TCR- $\alpha$ ) chains during T cell development in that recombination is terminated by a yet to be determined positive selection signal transduced by the BCR (81). Developing B cells remain in the pre-B cell compartment for a few hours whereas developing T cells remain in the CD4<sup>+</sup>CD8<sup>+</sup> double-positive compartment for 3–4 days (65, 82, 83). This kinetic difference may explain why Ig light chains are allelic excluded in B cells whereas TCR- $\alpha$  chains in T cells are not.

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