

Mutations in Ig α (CD79a) result in a complete block in B-cell development

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Mutations in Btk, μ heavy chain, or the surrogate light chain account for 85–90% of patients with early onset hypogammaglobulinemia and absent B cells. The nature of the defect in the remaining patients is unknown. We screened 25 such patients for mutations in genes encoding components of the pre-B-cell receptor (pre-BCR) complex. A 2-year-old girl was found to have a homozygous splice defect in Ig α , a transmembrane protein that forms part of the Ig α /Ig β signal-transduction module of the pre-BCR. Studies in mice suggest that the Ig β component of the pre-BCR influences V-DJ rearrangement before cell-surface expression of μ heavy chain. To determine whether Ig α plays a similar role, we compared B-cell development in an Ig α -deficient patient with that seen in a μ heavy chain-deficient patient. By immunofluorescence, both patients had a complete block in B-cell development at the pro-B to pre-B transition; both patients also had an equivalent number and diversity of rearranged V-DJ sequences. These results indicate that mutations in Ig α can be a cause of agammaglobulinemia. Furthermore, they suggest that Ig α does not play a critical role in B-cell development until it is expressed, along with μ heavy chain, as part of the pre-BCR.

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Introduction

The pre-B-cell receptor (pre-BCR) complex is expressed transiently and at very low cell density on the surface of developing B cells, yet it plays a pivotal role in B-cell production. The successful expression of the pre-BCR marks the transition of the pro-B stage to the pre-B stage of differentiation. In addition to the membrane form of a rearranged μ heavy chain, the complex includes 2 proteins that make up the surrogate light chain (VpreB and $\lambda 5$) and 2 proteins that comprise the transmembrane signal-transduction module (Ig α and Ig β). The surrogate light chain assesses the ability of the rearranged μ heavy chain to bind conventional light chain before the rearrangement of the light chain genes, and the Ig α /Ig β heterodimer masks the hydrophilic transmembrane domain of μ heavy chain and escorts it to the cell surface. In both humans and mice, the components of the surrogate light chain and the Ig α /Ig β heterodimer are expressed in the cytoplasm of B-cell precursors before the completion of V-DJ rearrangement (1, 2). This expedites cell-surface expression of the pre-BCR once a correctly recombined μ heavy chain has been produced.

In mice, the Ig α /Ig β heterodimer is also expressed on the surface of pro-B cells in the absence of μ heavy chain (3). Cross-linking of this receptor in mice that are unable to rearrange μ heavy chain genes induces tyrosine and serine/threonine phosphorylation of cytoplasmic proteins, and the differentiation of pro-B cells into pre-B cells (4). Furthermore, mice that do not make the Ig β

component of the signal-transduction module have normal D-J rearrangement but impaired V-DJ rearrangement (5). These findings suggest that signaling through the Ig α /Ig β heterodimer might facilitate heavy chain rearrangement, in a manner similar to the enhanced rearrangement of light chain genes seen after successful cell-surface expression of a rearranged μ heavy chain product as part of the pre-BCR (6, 7).

The Ig α and Ig β proteins, which are encoded by *mb-1* and *B29* genes (8, 9), are structurally similar to CD3 γ , δ , and ϵ chains on T cells. Each has a single extracellular immunoglobulin domain, a transmembrane domain, and an intracytoplasmic signaling domain with an immunoreceptor tyrosine-based activation motif (ITAM) (10). Glutathione-S-transferase fusion proteins of the cytoplasmic domains of Ig α and Ig β bind to distinct sets of effector molecules, and when transfected into cell lines, chimeric proteins containing the 2 cytoplasmic tails elicit different responses (11). Ig α tends to bind more readily to Src family members, and it activates tyrosine kinase more efficiently (11–13). However, when expressed in B cell-deficient mice as chimeric transgenes consisting of an IgM extracellular domain and an Ig α or Ig β intracellular domain, the signaling domains of both Ig α and Ig β are able to induce the pro-B cell to pre-B cell transition and allelic exclusion (14, 15). This suggests that the cytoplasmic domains of Ig α and Ig β are at least somewhat redundant in early B-cell development. This hypothesis is supported by observations in mice that lack the cytoplasmic

domain of Ig α . These mice have only a modest reduction in the number of pre-B cells and immature B cells, but markedly decreased numbers of mature B cells (16).

In broad strokes, early stages of B-cell development in the human correspond to those seen in the mouse; however, there are subtle differences that suggest some flexibility in the mechanisms used to regulate various stages of differentiation. In the mouse, defects in $\lambda 5$ cause a block in B-cell development at the pro-B to pre-B transition; however, this block is leaky, such that 4-month-old $\lambda 5$ -deficient mice have approximately 20% the normal number of B cells and they are able to make antibodies to T-dependent and T-independent antigens (17). By contrast, defects in $\lambda 5/14.1$ in humans result in a long-lasting, profound defect in B-cell development, such that an affected patient has less than 1% the normal number of B cells at 8 years of age (18). Early stages of B-cell development appear to be more dependent on IL-7 (19–21) and less dependent on Btk in mice compared with humans (22–25). To clarify better the nature of genetic defects that result in abnormal human B-cell development, we have screened patients with early-onset hypogammaglobulinemia and absent B cells for mutations in genes that are expressed in early stages of B-cell differentiation. We have identified a patient with a homozygous defect in Ig α production, and have compared the phenotype of this patient with that of a patient with defects in μ heavy chain.

Methods

Patient. The patient with Ig α deficiency was the first-born child of healthy parents without known consanguinity. The pregnancy and delivery were uneventful, but the patient developed recurrent diarrhea associated with failure to thrive within the first month of life. At 12 months of age, she was referred to Hacettepe University Hospital

for evaluation of bronchitis and neutropenia. At this time, her serum immunoglobulins were near the level of detection (IgG, 10 mg/dL; IgA, 13 mg/dL; and IgM, 5 mg/dL) and isohemagglutinins were absent. Analysis of peripheral blood lymphocytes revealed she had less than 1% CD19⁺ B cells, but normal numbers of T cells and natural killer cells and normal proliferation in response to mitogens. Intravenous γ -globulin therapy was initiated, and all symptoms resolved. At 2 years, 8 months of age, her physical examination was normal except for the absence of lymph nodes. Serum IgA and IgM were undetectable (< 5 mg/dL). The 13-year-old μ heavy chain-deficient patient included in this study was patient no. 7 in our previous report (26). The institutional review board of St. Jude Children's Research Hospital approved the study protocol, and informed consent was obtained from the parents.

Mutation detection. Genomic DNA was analyzed for mutations in Ig α by single-strand conformation polymorphism (SSCP) (27) using the primers indicated in Table 1. The following PCR conditions were used for all reactions: 95°C for 5 minutes followed by 30 cycles of 95°C for 45 seconds; the annealing temperature shown in Table 1 for 30 seconds; 72°C for 30 seconds with a final extension of 5 minutes at 72°C. Before SSCP analysis, PCR products from exons 1, 2, 3, and 5 were digested with *Pst*I, *Pst*I + *Mbo*I, *Mbo*I, and *Pvu*II, respectively. Fragments demonstrating altered mobility in SSCP were cloned into TA vector (Invitrogen Corp., Carlsbad, California, USA) and sequenced using M13 primers. The mutations were confirmed using a second independent PCR reaction.

Immunofluorescence staining. Peripheral blood lymphocytes and bone marrow cells were stained using techniques and reagents described previously (18).

Semiquantitative RT-PCR. Total RNA was extracted from 5×10^6 bone marrow mononuclear cells, using an RNeasy Mini kit (QIAGEN Inc., Valencia, California,

Table 1
PCR primers used to analyze early B-cell development

	Forward (5'-3')	Reverse (5'-3')	Annealing Temperature	Size of Fragment (bp)
Ig α genomic DNA				
Exon 1	TTGGGGTAGGGATCTGG	TGTGAGACTGTGGACGC	58°C	200+160 ^A
Exon 2	TTCTTCACTTTCCCGCATC	GGTACTGGCTCTATCCCC	60°C	260+181+129 ^A
Exon 3	AGGCTAGGGAGGGCAAGA	TTGGATGGGGAACCTCAG	58°C	151+122 ^A
Exon 4 (5')	GTAGACTCAGAGAGAACTG	ATATTTCATCCCCGGCATC	60°C	200
Exon 4 (3')	ACCCCTACAGAAACGATGGCAG	GAGCAGTCGTCCAGGTTCCAGG	66°C	229
Exon 5	AGGGTGTGATGTTCGCT	TAAGGAAGCCCCAGAAG	58°C	135+121 ^A
Ig α cDNA				
Exon 2	CGAGTCATACCAGCAGTC			
Exon 3		CGGCTGTGATGATTCGGT	56°C	107
Exon 5		GTCGTCCAGGTTCCAGGCC	56°C	250
Semiquantitative RT-PCR				
GAPDH	ATGCTGGCGCTGAGTAC	TGAGTCCTTCCACGATAC	56°C	257
Ig α	CGAGTCATACCAGCAGTA	GTCGTCCAGGTTCCAGGCC	56°C	250
Ig β	TCCGGCAATGTGAGCTG	ACCATCCTTCCAGCGTGT	56°C	218
TdT	TATGAGAGCAGCTTCTGTAAAG	TCTTTAACAGCAGCACACTGACG	60°C	375
RAG1	CCAGCTGTTTGTCTGGCCATCCGT	TTGGGATCTCATGCCTTCCAAGAT	60°C	353
VpreB	CATGCTGTTTGTCTACTGCACAG	TGCAGTGGGTTCCATTTCTTCC	66°C	396
$\lambda 5/14.1$	ACGCATGTGTTGGCAGC	GGCGTCAGGTTCCAGGTA	62°C	266
VDJ μ	GACACGGCCGTGTATTACTG	GGAATTCCTCACAGGAGACGA	56°C	150–200
VH1	GGGTACCATGGACTGGACCTGGAG	GGAATTCCTCACAGGACACGAG	50°C	450–500
VH3	GGGTACCATGGAGTTGGGCTGAG	GGAATTCCTCACAGGAGACGAG	60°C	450–500
VH4	GGGTACCATGAAACACCTGTGTTCTTC	GGAATTCCTCACAGGAGACGAG	60°C	450–500

^AAfter digestion with restriction enzyme.

USA), and cDNA was prepared with random hexamers and Superscript RT (GIBCO BRL, Gaithersburg, Maryland, USA). Semiquantification was performed by reference to amplification of GAPDH PCR reaction. To find semiquantitative range in each PCR reaction, preparatory PCR amplification from 18 to 33 cycles was performed. Each semiquantitative RT-PCR reaction includes serial 10-fold dilutions of control cDNA to show a quantitative amplification of template cDNA. PCR primers used for semiquantitative RT-PCR analysis are shown in Table 1. Oligonucleotide sequences of RAG1, TdT, VpreB, and V-DJ μ were derived from the previous publication (18), and RT-PCR of RAG1 was performed as described elsewhere (28). PCR conditions were as indicated here except for the addition of 3 μ Ci of [α - 32 P]dCTP. The PCR products were electrophoresed in 8% polyacrylamide gel and were viewed by autoradiography. To evaluate the VH repertoire, the PCR products were separated in a 6% denaturing polyacrylamide gel.

Results

Identification of mutation in Ig α . Genomic DNA samples from 25 patients with defects in B-cell development but no mutations in Btk (22, 23, 29), μ heavy chain (26), VpreB, or λ 5/14.1 (18) were analyzed by SSCP for mutations in Ig α and Ig β . DNA from a 2-year-old Turkish girl with agammaglobulinemia demonstrated an altered band migration in the analysis of exon 3 of Ig α (Figure 1a). Both parents of the child were heterozygous for this pattern, but analysis of DNA from 100 healthy controls did not reveal any additional samples with the same pattern, indicating that this was unlikely to be a polymorphism. This region of the gene was cloned and sequenced and a single-bp substitution (A \rightarrow G) at an invariant position (-2) of the splice acceptor site for exon 3 was revealed (Figure 1b).

The effects of this mutation on splicing of the Ig α message were evaluated by using RT-PCR. We amplified Ig α cDNA from the patient's bone marrow and bone marrow from healthy controls and another immunodeficient patient, using a primer from exon 2 paired with primers from either exon 3 or exon 5. When the exon 5 primer was used, the PCR product from the patient sample was approximately 120 bp smaller than the control PCR product. In addition, a faint PCR product that appeared smaller than the wild-type product was noted (Figure 2a). As expected, the sequence of the smaller PCR product from the patient demonstrated the deletion of exon 3. Because this exon encodes the transmembrane domain of Ig α and is 119-bp long, the deletion of this exon would be expected to result in a truncated protein that could not be expressed as part of the pre-BCR. To determine whether any intact transcripts were produced, the exon 2 primer was used with a primer from exon 3. The PCR product from this reaction demonstrated the deletion of the first 13 bp of exon 3 and the use of a cryptic splice site within exon 3 (Figure 2b). These results indicate that there were no wild-type Ig α transcripts in the patient's bone marrow.

Phenotypic analysis of B cells and B-cell progenitors. The consequences of mutations in Ig α on B-cell development were evaluated by immunofluorescence staining of

peripheral blood lymphocytes and bone marrow mononuclear cells. Like patients with mutations in μ heavy chain (26), the patient with defects in Ig α had undetectable CD19 $^{+}$ B cells (<0.01%) in the peripheral circulation. To determine the stage at which B-cell differentiation was blocked, bone marrow cells from the patient and an age-matched control were evaluated. For comparison, bone marrow from a patient with an amino acid substitution at an invariant cysteine in the CH4 domain of μ heavy chain was analyzed in parallel. This mutation would not be expected to affect V-DJ recombination, but it would prevent the normal folding and cell-surface expression of μ heavy chain. Both patients had markedly reduced percentages of CD19 $^{+}$ B-lineage cells (9% and 2%, respectively) when compared with the healthy control (46%) (Figure 3). Triple-color immunofluorescence was used to examine the ratio of pro-B cells

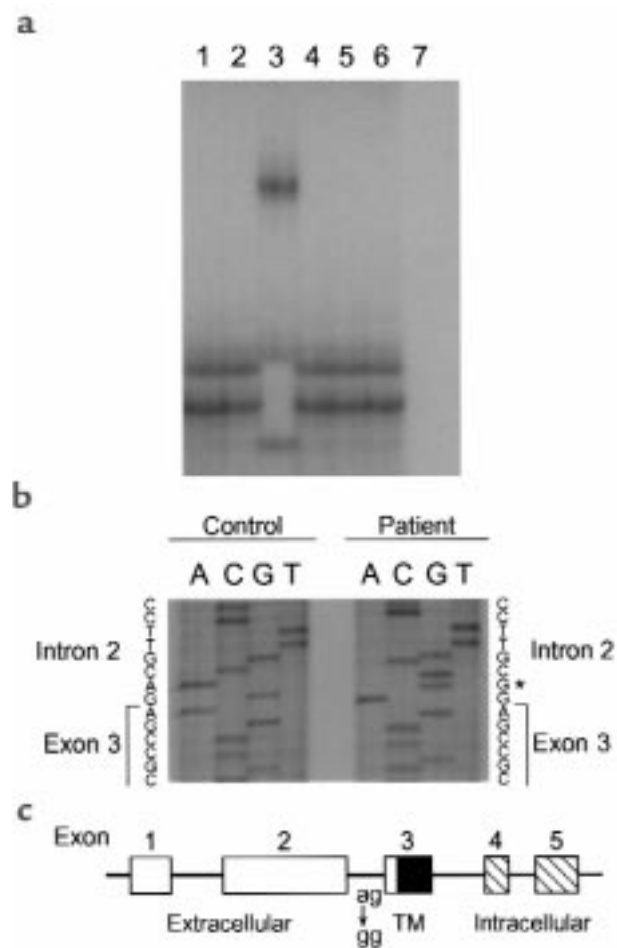
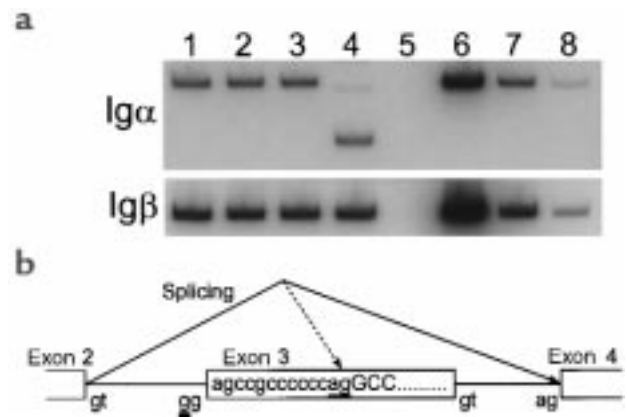


Figure 1

Identification of a mutation in the Ig α gene. (a) SSCP analysis of genomic DNA from a control (lane 1), patients with agammaglobulinemia (lanes 2-6), or no DNA template (lane 7) using primers specific for exon 3 of Ig α . The DNA from the patient is shown in lane 3. (b) Sequence analysis revealed a single-bp substitution (A \rightarrow G) at the invariant -2 position of the splice acceptor site for exon 3. The patient's mutation is noted by an asterisk. (c) Schematic diagram of the 5 exons constituting the genomic structure of Ig α . The extracellular region (open), the transmembrane region (TM; filled), the intracellular region (hatched), and the position of the point mutation in the patient are indicated.

Figure 2

Effects of the Ig α mutation on splicing. (a) Bone marrow-derived cDNA from 2 healthy controls (lanes 1 and 2), a patient with μ heavy chain deficiency (lane 3), and the patient with mutations in Ig α (lane 4) was amplified using primers from exon 2 and exon 5 of Ig α (top) or primers specific for Ig β (bottom). Ten-fold dilutions of control cDNA (10 \times , 1 \times , and 0.1 \times) are shown in lanes 6–8. Sequencing of the smaller band in lane 4 demonstrated skipping of exon 3 of Ig α . The sequence of the fainter band in this lane showed the use of a cryptic splice site within exon 3. (b) Schematic diagram showing exon skipping and activation of the cryptic splice site within exon 3. The coding sequence for exon 3 is shown within the central box. The majority of the Ig α transcripts amplified in lane 4 in panel (a) delete exon 3, as indicated by the solid black line. A fraction of the transcripts are derived from the use of the cryptic splice site within exon 3, indicated by the dotted line. The invariant dinucleotides at the splice donor and acceptor sites are shown. The position of the point mutation and the dinucleotide at the cryptic acceptor site are underlined. The intron and the coding sequence that arise from the use of the cryptic splice site are shown in lowercase letters and uppercase letters, respectively.



(CD19 $^{+}$, CD34 $^{+}$, sIg $^{-}$), pre-B cells (CD19 $^{+}$, CD34 $^{-}$, sIg $^{-}$), and B cells (CD19 $^{+}$, CD34 $^{-}$, sIg $^{+}$) in the 3 individuals. Within the CD19 $^{+}$ population, 27% of the cells from the healthy control expressed surface immunoglobulin, but less than 1% of the cells from either patient were sIg $^{+}$, confirming the severe block in B-cell differentiation. In both the patient with mutations in μ heavy chain and the patient with mutations in Ig α , more than 75% of CD19 $^{+}$ cells were pro-B cells, as defined by coexpression of CD34 $^{+}$, whereas in the normal control patient, only 12% of the CD19 $^{+}$ cells were CD34 $^{+}$. This finding suggested that the B-cell differentiation was blocked at the pro-B to pre-B transition in the 2 disorders. This block was more clearly demonstrated in permeabilized cells stained for CD19, TdT, and μ heavy chain (Figure 3). In both patients, more than 94% of the CD19 $^{+}$ cell were positive for TdT and negative for cytoplasmic μ heavy chain, a phenotype characteristic of pro-B cells. These findings demonstrate that B-cell development in Ig α deficiency is blocked at the pre-BCR checkpoint and that the block is identical to that seen in μ heavy chain deficiency.

Gene expression in B-cell progenitors. Semiquantitative RT-PCR was used to evaluate the expression of genes specific to various stages of B-cell differentiation. We included age-matched controls for each patient and analyzed transcripts directly linked to early B-cell development and immunoglobulin gene expression. To demonstrate that PCR amplification was in the linear range, three 10-fold dilutions of control cDNA were included in each assay. GAPDH control amplification showed that equivalent amounts of cDNA were used in each RT-PCR amplification. The transcripts associated with the pro-B stage — TdT, RAG1, VpreB, and λ 5/14.1 transcripts — were expressed in approximately equal amounts in the patient with Ig α deficiency, the one with μ heavy chain deficiency, and the healthy controls (Figure 4a). However, the amount of V-DJ rearranged- μ heavy chain transcripts were greatly decreased and equivalent in the patient with Ig α deficiency and in the patient with μ heavy chain deficiency (Figure 4b). This finding was further confirmed by using VH3-, VH4-, and VH1-specific primers to evaluate the use of each of these VH families.

To examine the diversity of the repertoire of rearranged μ heavy chains, the VH-specific PCR products were separated on a denaturing 6% polyacrylamide (sequencing) gel to allow the discrimination of transcripts with CDR3 regions of varying length (30). To avoid overloading, the control samples were diluted by a factor of 10 compared with the patient samples. Control bone marrow samples showed a diverse repertoire, which was characterized by more than 15 bands separated from each other by 3 bp (Figure 5). The bands showed a Gaussian distribution in size with intensity of a band correlating with the total amount of μ heavy chain CDR3 transcripts of that length. In both the Ig α -deficient patient and the μ heavy chain-deficient patient, there were fewer bands and each band was faint and of equal intensity. The diversity of repertoire was approximately equal in the 2 patients. The degree of diversity in the 2 patients was further compared in experiments using the VH1-, VH3-, and VH4-specific primers. In every experiment, transcripts could be amplified from each patient, but the repertoire was limited. The VH-specific PCR reactions were performed 4 times, and although the repertoire varied slightly in each experiment, the number of bands was always similar in the samples from the patient with mutations in Ig α and the patient with mutations in μ heavy chain. These results demonstrate that V-DJ rearrangement in the patient with mutations in Ig α was not decreased compared with that seen in a patient with an amino acid substitution in the constant region of μ heavy chain. When coupled with the phenotypic studies, these results confirm that the block in B-cell differentiation in Ig α deficiency is at the pre-BCR checkpoint and is identical to that seen in patients with defects in μ heavy chain.

Discussion

The majority of patients with early-onset hypogammaglobulinemia and absent B cells are males with X-linked agammaglobulinemia (29). These patients have mutations in the hematopoietic-specific cytoplasmic tyrosine kinase Btk (22, 23, 29). The remaining patients appear

to have a heterogeneous group of disorders. We have recently shown that some of these patients have mutations in μ heavy chain (26) or $\lambda 5/14.1$ (18), both of which are components of the pre-BCR complex (31, 32). To determine whether defects in other components of the pre-BCR might result in immunodeficiency, we screened DNA from affected patients for mutations in VpreB, Ig α , and Ig β . We identified a 2-year-old girl with a homozygous splice defect resulting in the truncation of Ig α upstream of the transmembrane domain.

Like patients with defects in μ heavy chain (26), the patient with mutations in Ig α had no detectable CD19⁺ B cells in the peripheral circulation. Analysis of bone marrow from this patient and a patient with an amino acid substitution in μ heavy chain showed that both patients had normal numbers of pro-B cells but an almost complete absence of pre-B cells. There is considerable variation in the number of pro-B cells in normal individuals, and the number tends to decrease with age. This may explain the slightly higher number of pro-B cells in the patient with Ig α deficiency compared with the patient with μ heavy chain defects. The amount and diversity of μ heavy chain V-DJ rearrangements in these 2 disorders were equivalent. Both patients had a small number of transcripts utilizing members of the VH1, VH3, and VH4 families.

Mice that are null for Ig α have not been described; however, mice with a truncation in Ig α that removes the COOH-terminal 40 amino acids of the cytoplasmic domain, including the ITAM motif, have a relatively mild block in the pro-B to pre-B transition (16). This suggests that the complete failure of B-cell development in the patient with a truncation in Ig α before the transmembrane domain is due to the inability of this protein to escort the μ heavy chain and the Ig β component of the signal-transduction module to the cell surface, rather than the loss of the Ig α signaling capacity.

In contrast to our findings in the Ig α -deficient patient, which show a block at the pro-B to pre-B transition similar to that seen in μ heavy chain deficiency, studies in mice with defects in Ig β are reported to show decreased V-DJ rearrangement when compared with μ -deficient mice (5). There are several possible explanations for the discordance between human Ig α deficiency and murine Ig β deficiency. Under some circumstances, Ig β may reach the cell surface in the absence of Ig α (33). If homodimeric complexes of Ig β (34), but not Ig α , are expressed, it might be possible for Ig β to transmit signals in the absence of Ig α , whereas the reverse would not be true. Alternatively, it is possible that there are signal-transduction pathways that are initiated in the cytoplasm by the ITAM-containing domain of Ig β but not Ig α . Finally, despite

the overall similarity in early B-cell development in mice compared with humans, there may be differences between the 2 species in the cell-surface expression or signaling capacity of Ig α /Ig β complexes.

Complexes containing the Ig α /Ig β heterodimer can be detected on the surface of murine B-cell precursors before V-DJ rearrangement (3); however, attempts to document the expression of the same complex on human pro-B cells have not been successful (2, 3, 35, 36). It should be noted that it has been more difficult to demonstrate the cell-surface expression of all the components of the pre-BCR in human B-cell precursors compared with murine counterparts. In mice, surrogate light chains form complexes with surrogate heavy chains and can be identified on the surface of pro-B

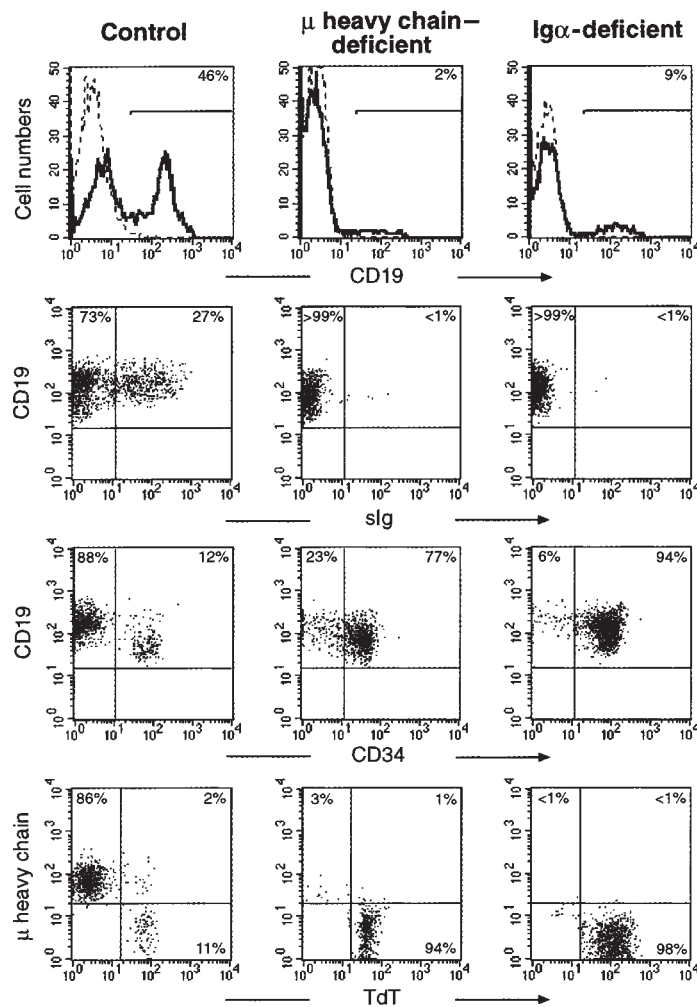


Figure 3

Flow cytometric analysis of B-cell development. Bone marrow mononuclear cells from a control (left column), μ heavy chain-deficient patient (middle column), and Ig α -deficient patient (right column) were stained with antibody to CD19. Both patients showed a marked reduction in the number of CD19⁺ cells within the lymphoid gate (first row). Less than 1% of the CD19⁺ cells from either patient were B cells, as defined by coexpression of CD19 and surface immunoglobulin (second row). More than 75% of the B-lineage cells in both patients were positive for CD34 (third row). CD19⁺ permeabilized cells were stained for cytoplasmic TdT and μ heavy chain. More than 94% of the CD19⁺ cells from both patients were pro-B cells, as defined by coexpression of CD19 and TdT (fourth row).

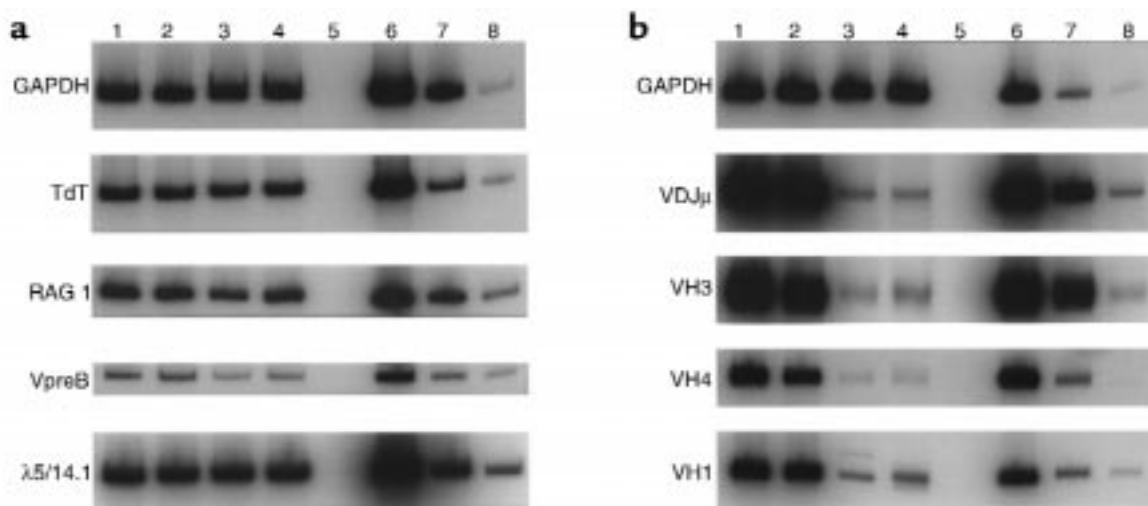


Figure 4

Semiquantitative RT-PCR analysis of B cell-specific transcripts in the bone marrow. (a) Equal amounts of cDNA from 2 healthy controls (lanes 1 and 2), a patient with μ heavy chain deficiency (lane 3), the patient with $Ig\alpha$ deficiency (lane 4), a cDNA negative template control (lane 5), and three 10-fold dilutions of control cDNA (10 \times , 1 \times , and 0.1 \times) (lanes 6–8) were amplified using primers specific for TdT, RAG1, VpreB, and $\lambda 5/14.1$. GAPDH was used as a control to demonstrate equal concentrations of cDNA. (b) cDNA samples were analyzed as in a, except that the 10-fold dilutions in lanes 6–8 were 1 \times , 0.1 \times , and 0.01 \times of the control. Primers specific for all VH family members or primers specific for VH3, VH4, or VH1 were paired with a primer from the CH1 domain of μ heavy chain.

cells in the absence of μ heavy chains (37). There is some controversy about the existence of similar complexes on human pro-B cells. Some groups have evidence suggesting that these complexes are present (38–40) but others maintain that these complexes do not occur (41). Finally, it appears that the intact pre-BCR is expressed more transiently and at lower intensity in human B-cell precursors compared with murine precursors (1, 42). Although these differences have sometimes been attributed to the reagents or techniques used to demonstrate the various proteins, true differences between the 2 species may exist.

Murine models of immunodeficiency, in which single genes have been rendered nonfunctional, have dramatically increased our understanding of normal lymphocyte development. However, it is clear that there is not always complete concordance between mice with defects in a particular gene and patients with mutations in the same gene. Detailed evaluation of patients with single gene defects of the immune system can elucidate

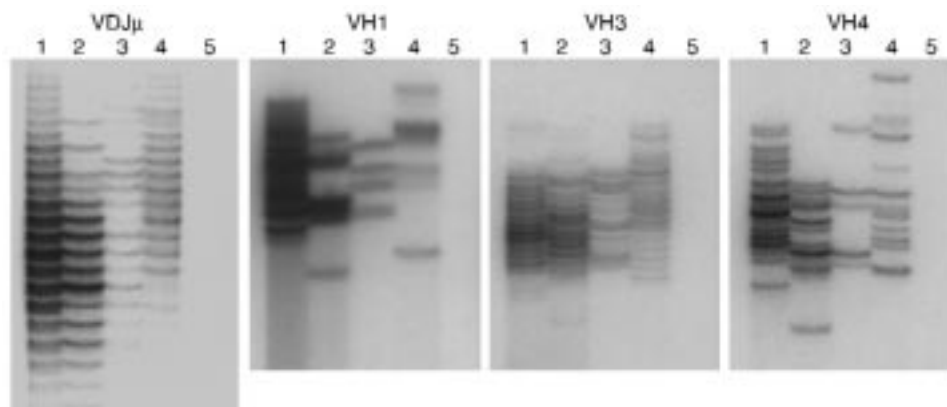
the pathways in lymphocyte differentiation that are invariant and highly consistent between species and those that show some flexibility and may vary from 1 species to another. In this study, we have done a side-by-side comparison of B-cell differentiation in 2 patients with defects in different components of the pre-BCR. A patient with a functionally null defect in $Ig\alpha$ and a patient with a defect in μ heavy chain that would prevent cell-surface expression of the antigen receptor complex had an identical block in B-cell differentiation. This indicates that in the human, $Ig\alpha$ is essential for the expression of the pre-BCR, but, does not appear to play a role in B-cell development until it is expressed along with μ heavy chain as part of the pre-BCR.

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Figure 5

Repertoire diversity of V-DJ rearranged μ heavy chain transcripts. RT-PCR products from Figure 4 were separated on a denaturing 6% polyacrylamide (sequencing) gel. The control samples (lanes 1 and 2) were diluted 10-fold compared with the patient samples. PCR products from the μ heavy chain-deficient patient are shown in lane 3, and products from the $Ig\alpha$ -deficient patient are shown in lane 4.



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