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CUTTING EDGE

Cutting Edge: A Hypomorphic Mutation in Igβ (CD79b) in a Patient with Immunodeficiency and a Leaky Defect in B Cell Development[1]

A. Kerry Dobbs,[*] Tianyu Yang,[*] Dana Farmer,[*] Leo Kager,[†] Ornella Parolini,[‡] and Mary Ellen Conley[2][§]*

***Although null mutations in Igα have been identified in patients with defects in B cell development, no mutations in Igβ have been reported. We recently identified a patient with a homozygous amino acid substitution in Igβ, a glycine to serine at codon 137, adjacent to the cysteine required for the disulfide bond between Igα and Igβ. This patient has a small percentage of surface IgM^{dim} B cells in the peripheral circulation (0.08% compared with 5–20% in healthy controls). Using expression vectors in 293T cells or Jurkat T cells, we show that the mutant Igβ can form disulfide-linked complexes and bring the μ H chain to the cell surface as part of the BCR but is inefficient at both tasks. The results show that minor changes in the ability of the Igα/Igβ complex to bring the BCR to the cell surface have profound effects on B cell development.** The Journal of Immunology,* 2007, 179: 2055–2059.

Cell surface expression of the pre-BCR and BCR varies considerably throughout B cell differentiation. In pre-B cells, the pre-BCR is present at very low density (1, 2). Immature B cells, as seen in the neonate, have high density surface IgM, and more mature B cells have lower density surface IgM in both the human and the mouse (2). The factors that control the amount of the receptor that can be found on the cell surface are not well understood and the effects of altering the expression have not been fully evaluated.

It is clear that the signal transduction molecules Igα and Igβ (CD79a and CD79b), play a critical role in BCR expression (3). These two proteins function as a disulfide-linked heterodimeric complex that escorts the μ H chain to the cell surface. Both Igα and Igβ consist of an extracellular Ig domain, a membrane proximal spacer region containing the cysteine required for the interchain disulfide link, a transmembrane domain, and a cytoplasmic domain containing a single ITAM motif (3). Loss of Igα or Igβ in knockout mice or in patients with null mutations in Igα result in a complete block at the pro-B cell to pre-B cell transition (4–6). Similarly, mice that have a truncated Igβ lacking the ITAM motif and an Igα in which the tyrosine residues in the ITAM have been mutated to phenylalanine have a complete block at the pro-B cell to pre-B cell stage of differentiation (7). Changes in the extracellular domains of Igα and Igβ have not been evaluated as extensively. However, Siegers et al. have shown that mutation of the Igα extracellular cysteine that is required for the interchain disulfide bond results in a protein that can be expressed inefficiently as part of a BCR in a plasma cell (8). Expression of the BCR containing the mutant Igα was ∼40% of that seen with wild-type Igα.

We have identified a patient with a homozygous amino acid substitution, a glycine to serine at codon 137, in the membrane proximal spacer region of Igβ. This patient had the early onset of infection, profound hypogammaglobulinemia, and markedly reduced but not absent B cells. This suggests that the membrane proximal spacer regions of Igα and Igβ have a critical role in the assembly or function of the BCR.

Materials and Methods

Patients

The patients included in this study were analyzed as part of a research study approved by the St. Jude Children's Research Hospital Institutional Review Board (Memphis, TN). Inclusion criteria for the study included the onset of infections at <5 years of age, hypogammaglobulinemia, and $<2\%$ CD19 B cells in the peripheral circulation.

Mutation detection

Genomic DNA was isolated from whole blood or activated T cells and analyzed by single-stranded conformational polymorphism (SSCP)[3] using previously described techniques (9). The primers for SSCP were designed to flank the six exons and associated flanking splice sites (sequence available on request) of Igβ. Exon 3 from the patient was cloned and sequenced as previously described (6).

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[3] Abbreviations used in this paper: SSCP, single-stranded conformational polymorphism; MSCV, mouse stem cell virus; YFP, yellow fluorescent protein.

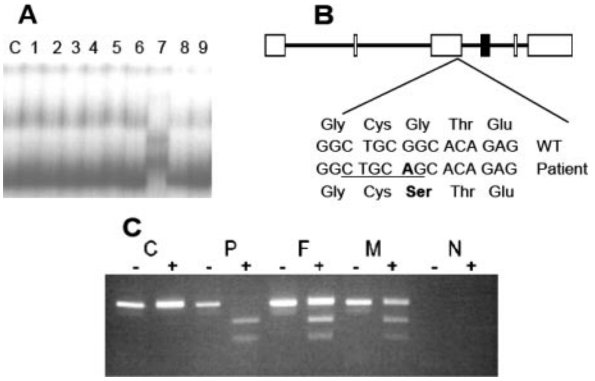

FIGURE 1. A homozygous mutation in Igβ in a patient with immunodeficiency. *A*, Samples of genomic DNA from a healthy control (*lane C*) and from nine patients with markedly reduced or absent B cells were analyzed by SSCP for alterations in exon 3 of Igβ. *Lane 7* contains DNA from the patient. *B*, Schematic diagram of the six exons that encode Igβ. The exon encoding the transmembrane domain, exon 4, is shown in black. The single base pair substitution in codon 137 and the amino acid substitution are shown in bold type. The *Pst*I site in the DNA from the patient is underlined. *C*, Exon 3 of Igβ from a control (*lane C*), the patient (*lane P*), the patient's father (*lane F*), the patient's mother (*lane M*), or a DNA negative control (*lane N*) was amplified by PCR and the product was left untreated (−) or digested with *Pst*I (+).

Immunofluorescence staining

Peripheral blood lymphocytes were separated by Ficoll Hypaque centrifugation, washed, resuspended at a final concentration of 10^7 cells/ml and distributed into staining tubes with 5×10^5 cells/tube. Staining was done in the presence of 50 μl of rabbit IgG (DakoCytomation) to block nonspecific staining. The FITC-labeled goat anti-human IgM, and anti-CD21 were obtained from Southern Biotechnology Associates. The PE-labeled anti-CD19 and FITC-labeled CD22 were obtained from BD Biosciences. The PE-labeled anti-CD38 was obtained from Beckman Coulter. Cells were stained 15 min on ice in the dark and then washed twice. After the final wash, cells were resuspended in 0.5% paraformaldehyde and analyzed on a BD FACScan within 24 h.

Cells

The Jurkat T cell lines were maintained in RPMI 1640 supplemented with 15% FCS, 2 mM L-glutamine, 50 μM 2-ME, and 20 μg/ml ciprofloxacin. Human embryonic kidney fibroblast 293T cells were cultured in DMEM with 10% FCS, 1.5 g/L sodium bicarbonate, 2 mM L-glutamine, and 20 μg/ml ciprofloxacin.

Western blotting

Cells were lysed in buffer containing 1% digitonin, 50 mM Tris (pH 7.6), and 150 mM NaCl. The lysates were separated on 9% SDS-PAGE minigels and then transferred to polyvinylidene fluoride membranes. Blots were developed with mAbs to IgM, the λ L chain, and Igβ from Southern Biotechnology Associates and a mAb to Igα from Santa Cruz Biotechnology.

Retroviral vector construction, virus production, and gene transduction

Murine stem cell virus (MSCV) retroviral vectors containing an internal ribosomal entry site and either GFP or yellow fluorescent protein (YFP) were used as the backbone for the expression vectors (10). A cassette containing the sequence for a wild-type λ L chain and μ H chain linked by the A2 self-cleaving peptide (11) was placed in the GFP-containing vector. The YFP vector was used to produce a vector containing wild-type Igα (CD79a) followed by the A2 sequence and either wild-type or mutant Igβ (CD79b).

Retroviral production and Jurkat transduction were performed as previously described (12) with minor modifications. Briefly, 10^6 293T cells in 10-cm tissue culture dishes were cotransfected with 2 μg of pRD114 (envelope protein from a feline endogenous virus), 4 μg of pEQ-PAM3 (*gag*/*pol* plasmid), and 4 μg of the experimental GFP or YFP vector in the presence of a FuGENE transfection reagent. After 24 h the medium was replaced and the virus-containing medium was harvested 48 and 72 h after transfection. The virus was snap frozen on dry ice and stored at −80°C. The virus was titered in 293T cells.

Results and Discussion

Samples of genomic DNA from patients with the early onset of infection, profound hypogammaglobulinemia, and markedly reduced or absent peripheral blood B cells were screened by SSCP for mutations in Igβ. In the analysis of exon 3, DNA from one patient demonstrated abnormal fragments with the loss of the normal fragments (Fig. 1*A*). This region was cloned and sequenced and a single base pair substitution, a G to A, was identified in codon 137. This alteration results in the replacement of the wild-type glycine with serine at a position that is immediately downstream of the cysteine that forms the disulfide bridge with Igα in the proximal membrane spacer region (Fig. 1*B*). The wild-type glycine at this site is conserved not only in Igβ from humans, mice, dogs, and cattle but also in Igα from humans, mice, dogs, and cattle.

The G to A substitution in this patient results in the creation of a *Pst*I restriction site that was used to analyze the patient's parents and 100 normal controls. As shown in Fig. 1*C*, the patient's parents were heterozygous for the mutation; however the alteration was not seen in the 100 normal controls.

The patient with the Igβ mutation is a 15-year-old girl of Georgian (South Caucus) descent living in Austria. She was well until 5 mo of age when she developed recurrent bronchitis. At 15 mo of age she was evaluated because of persistent cough and pneumonia and was found to have panhypogammaglobulinemia (IgG at 84 mg/dl (normal 286-1680 mg/dl), IgM at 29 mg/dl (normal 26–218 mg/dl), and IgA at 15 mg/dl (normal 19–220 mg/dl)) and <1% B cells. She was started on i.v. γ-globulin at that time. She was treated for pneumonia at 3 years of age and 10 years of age. Currently she is doing well on s.c. γ-globulin with normal growth and development and no signs of infection. On her most recent evaluation she had a serum IgG of 617 mg/dl, IgM of <4 mg/dl, and IgA of <5 mg/dl.

Bone marrow was not available from the patient; however, peripheral blood studies showed that the amino acid substitution resulted in a leaky defect in B cell development. The patient did have a small number of $CD19^+$ B cells in the peripheral circulation (0.08% compared with 5–20% in normal controls). In healthy controls the intensity of CD19 expression by FACS analysis is relatively uniform, whereas the patient's B cells were variable in intensity of CD19 with approximately half of the cells being dimmer than is typical (Fig. 2). The amount of surface IgM was below the threshold of detection; however the cells that were brighter for CD19 appeared to have more surface IgM than the $CD19^{dim}$ cells. The $CD19^{dim}$ cells were positive for CD38 and negative for CD21 and CD22. The $CD19^{bright}$ cells were negative for CD38 and positive for CD21 and CD22. With the exception of surface IgM staining, this pattern is similar to that seen in immature B cells and in patients with mutations in Btk (X-linked agammaglobulinemia). The amount of Igβ cDNA in the peripheral blood cells of the patient was similar to that seen in the patient with a mutation in Btk. Both were ~1% of that seen in the controls (data not shown).

Both Igβ and Btk are required for signaling through the BCR at all stages of B cell differentiation. The similarities in the phenotype of the B cells that are produced in the patient with a hypomorphic mutation in Igβ and patients with mutations in Btk suggest that the abnormal B cell phenotype seen in Btk-deficient patients can be attributed to faulty signaling through

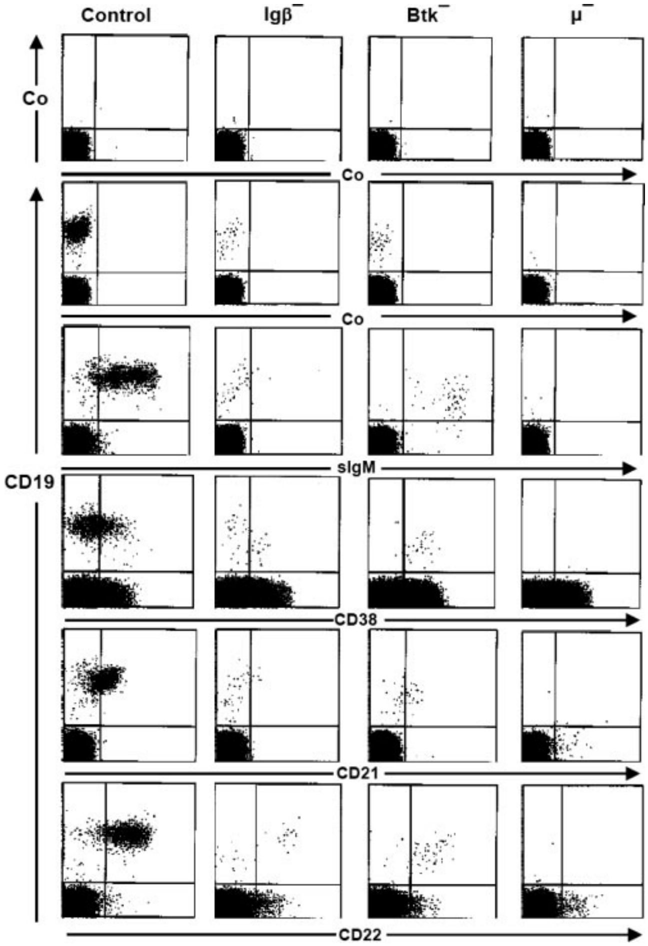

FIGURE 2. B cell phenotype in a patient with defects in B cell development. Ficoll density separated peripheral blood lymphocytes were stained with isotype control Abs (*top row*) or with PE-labeled CD19 and FITC-labeled surface IgM, CD38, CD21, or CD22. Cells from a healthy control (*left column*), the patient with a mutation in Igβ (*second column from left*), an 11 year old patient with a premature stop codon (R255X) in Btk (*third column from left*), and a 5-year-old patient with a large deletion of the μ constant region on one allele and a 2-bp deletion (AA del in codon 168) in exon 2 of the μ H chain on the other allele (*right column*) are shown. The number of gated events shown is 17,000–19,000 in the healthy control sample and 100,000–150,000 in the patient samples.

the BCR rather than abnormal function of other pathways that use Btk. The low or absent expression of surface IgM in the patient with the Igβ mutation indicates that this defect strongly impairs cell surface expression of the BCR.

To examine the functional consequences of the amino acid substitution in Igβ in an in vitro system, retroviral expression vectors that would allow the production of an artificial BCR were assembled. A construct containing a wild-type μ H chain (VH3-23 with no somatic mutations) and wild-type λ L chain linked by a self-cleaving A2 sequence was inserted into a GFP-producing MSCV vector. The sequence encoding wild-type Igα and either wild-type or mutant Igβ, linked by an A2 sequence, was ligated into a YFP-producing MSCV vector. 293T cells were transfected with the μ/λ vector and either the wild-type or the mutant Igα/Igβ vector. Cell lysates were obtained 20 h later and analyzed by Western blotting. As shown in Fig. 3*A*, the mutant Igβ protein was stable and migrated similarly as the wild-type Igβ in a reducing gel.

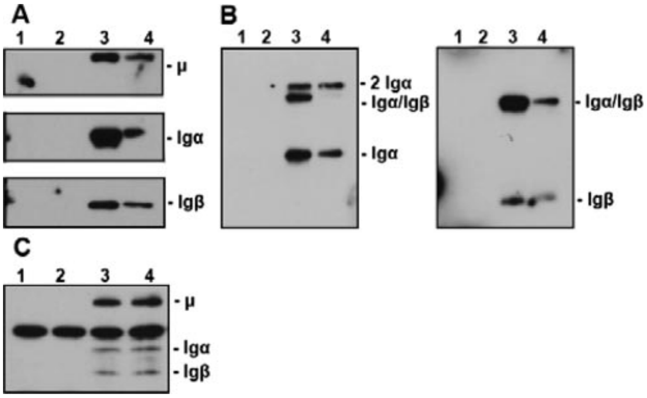

FIGURE 3. The mutant Igβ is stable but inefficient in forming disulfide bonds with Igα. 293T cells were untreated (*lane 1*) or transfected with empty retroviral vectors (*lane 2*) or with a retroviral vector expressing the μ H chain and the λ L chain and a vector containing Igα and either wild-type (*lane 3*) or mutant Igβ (*lane 4*). *A* and *B*, Total cell lysate was separated in reducing gels and developed with Abs to IgM, Igα, or Igβ (*A*) or in nonreducing gels and developed with Abs to Igα (*left*) or Igβ (*right*) (*B*). *C*, BCR complexes were immunoprecipitated with Abs to IgM and run on reducing gels and then analyzed with Abs to IgM, Igα, and Igβ.

The ability of the mutant Igβ to form disulfide bonds with Igα was analyzed in nonreducing gels. Approximately half of the wild-type Igα was found in high m.w. complexes containing Igα dimers or Igα/Igβ heterodimers in the cells containing the wild-type Igβ. By contrast, almost none of the Igα was complexed with Igβ in the cells containing the mutant Igβ. Nonreducing blots probed with anti-Igβ showed that the majority of the wild-type Igβ was in high m.w. complexes; however a much smaller fraction of the mutant Igβ was found in the dimeric complexes. The ability of the mutant Igβ to combine with the μ H chain and Igα was assessed by immunoprecipitation. When the cell lysate was immunoprecipitated with an anti-μ Ab the amount of Igα and Igβ that coprecipitated was equal in the cells containing either the wild-type or the mutant Igβ. These results indicate that the mutant Igβ can form disulfide-linked complexes with Igα, but this process is inefficient.

Cell surface expression of the mutant Igβ was examined in a more physiologic system, stably transduced Jurkat T cells. Cells were transduced with empty GFP and YFP vectors, vectors containing wild-type components of the BCR, or vectors containing wild-type μ, λ, and Igα, with Igβ bearing the amino acid substitution at codon 137. Six to 10 days after transduction, GFP$^+$YFP$^+$ cells were sorted and placed back into culture. The cultured cells were stained for surface expression of IgM 8 to 30 days after the sort. Although the expression of GFP and YFP was comparable in the cells containing components of the wild-type BCR and cells containing the mutant Igβ, the cells containing the mutant Igβ had decreased expression of surface IgM. There were fewer cells that were positive for IgM, and the cells that were positive for IgM were brighter for GFP and YFP (Fig. 4), providing further support for the contention that the mutation in Igβ impairs assembly and cell surface expression of the BCR. Our results clearly show that mutations in Igβ can cause a profound defect in B cell development. The severe block in B cell differentiation seen in our patient might be considered surprising in view of the relatively conservative change from glycine to serine and the observation that the mutant Igβ could be

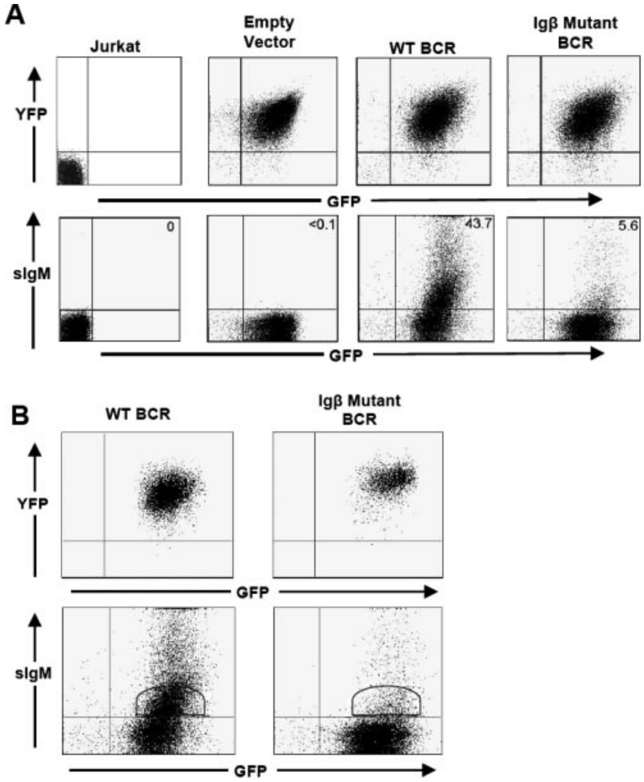

FIGURE 4. The mutant Igβ allows inefficient expression of cell surface BCR. Untreated Jurkat cells were cultured in parallel with cells that had been transduced with empty GFP and YFP vectors, cells that had been transduced with GFP and YFP vectors containing components of the wild-type BCR (WT BCR), and cells transduced with GFP and YFP vectors containing wild-type μ, λ, and Igα and mutant Igβ. *A*, The intensity of GFP and YFP in the transduced cell lines is shown in the *top panel*. The percentage of cells positive for surface IgM is shown in the *lower panel* and noted in the *upper right corner* of each plot. *B*, A gate was drawn on the surface IgM-positive cells and the intensity of GFP and YFP is shown. The mean GFP fluorescence intensity for the WT BCR and the Igβ mutant BCR was 318 and 617 respectively; the mean YFP intensity for the same cells was 211 and 462. The experiment shown is representative of three sets of transductions.

incorporated into the BCR in Jurkat T cells. However, the position of this glycine, adjacent to the cysteine required for the disulfide bridge, suggests that structural constraints may not permit any substitutions at this site. Notably, the decrease in expression of the mutant BCR in the Jurkat cell line is similar to the decreased expression of a BCR containing Igα with a mutation in the cysteine required for the interchain disulfide bond in a plasma cell line (8).

Siegers et al. found that Igα with a mutation of the cysteine required for the disulfide bond could be expressed normally as part of the BCR in insect cells (8). Similarly, we found that transient transfection of either 293T cells or Jurkat cells with components of a BCR that included the mutant Igβ allowed expression of a cell surface BCR equivalent to that seen when a wild-type Igβ was used (data not shown). It is probable that the amounts of protein produced in transient transfection or in nonmammalian cells overcomes the inefficiency of the BCR assembly. Less protein is produced by the integrated retroviral vectors in the Jurkat system, a system that may more accurately mimic the endogenous production of the pre-BCR.

Because the pre-BCR is expressed at very low cell surface density, impaired efficiency of expression of this receptor may result in a cell surface density that falls below the threshold required to initiate the pro-B cell to pre-B cell transition or the expansion of the pre-B cell population. In our patient, it is likely that only a small number of cells move through this bottle neck and those that do mature into B cells are not able to expand or function because of the low cell density of the BCR.

Our study adds Igβ to the list of gene defects that can result in a failure of B cell development in patients with immunodeficiency. The majority of patients with the early onset of infection, panhypogammaglobulinemia, and markedly reduced or absent B cells (over 85%) are males with mutations in Btk (X-linked agammaglobulinemia) (13). As noted above, mutations in Btk result in a leaky defect in B cell development such that the majority of affected patients have a small number of B cells in the peripheral circulation and a measurable amount of serum IgG at the time of diagnosis. The B cells that are present have a distinctive phenotype with variable intensity expression of CD19 but high expression of surface IgM (14, 15). The extended phenotype, characterized by increased expression of CD38 and decreased expression of CD21, has been seen in over 50 patients with X-linked agammaglobulinemia (A.K. Dobbs and M.E. Conley, unpublished studies).

Approximately 5% of patients with defects in B cell development but no other findings have defects in the μ H chain (16), and a small number have defects in λ5 (17), Igα (6), or the B cell linker protein BLNK (18). The majority of these patients have null mutations that completely ablate the function of the associated protein. Hypomorphic mutations, as seen in the subject of this report, can provide valuable insight into the assembly and function of the BCR.

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Disclosures

The authors have no financial conflict of interest.

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