

Ig β deficiency in humans

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Purpose of review

To describe novel immunological and molecular findings regarding early B cell development arrest resulting in autosomal recessive agammaglobulinemia.

Recent findings

Recently two different groups identified mutations in Ig β , a component of the pre-B cell receptor, responsible for agammaglobulinemia in humans. These are the first two patients ever described with mutations in Ig β .

Summary

These novel findings broaden the spectrum of genetic defects underlying this rare condition. This novel cause of agammaglobulinemia not only sheds light into early B cell development in humans but also sets the basis for potential alternative therapeutic approaches such as gene therapy.

Keywords

agammaglobulinemia, B cell development, Ig β , pre-B cell receptor

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Introduction

Agammaglobulinemia is a rare congenital immunodeficiency characterized by absence of circulating B cells and low or absent serum immunoglobulin levels [1]. Most affected patients are men and mutations in the gene encoding Btk, a tyrosine kinase, have been identified as the underlying cause in 85–90% of cases [2]. In the remaining patients affected with the autosomal recessive form of the disease, mutations in components of the pre-B cell receptor (BCR), such as μ heavy chain (μ HC), lambda5, Ig α , or downstream signaling elements have been described to cause the disease [3–6,7*]. This review focuses on the recent identification of mutations in the gene encoding Ig β resulting in agammaglobulinemia.

Early B cell development

Early B cell development is a highly regulated process that takes place initially in the embryonic liver and then in the bone marrow [8]. Initially, V(D)J recombination gives rise to a rearranged μ HC, which is the first isotype to be produced. If this μ HC is able to pair with the surrogate light chain (SLC), formed by VpreB and lambda5, the pre-BCR complex is expressed on the surface of B cell precursors [9–15]. The pre-BCR complex includes the signaling heterodimer composed of Ig α /Ig β . Animal knockout models for components of the pre-BCR, such as μ HC, lambda5, and Ig α have demonstrated that the expression of this complex is essential for the transition from pro-B to pre-B cells. Mutation analysis of

patients affected with the autosomal recessive form of agammaglobulinemia confirmed the importance of the pre-BCR in early B cell development in humans as mutations in μ HC, Ig α , lambda5, or downstream signaling elements such as BLNK have been identified to be causative of this condition. If the pre-BCR is normally expressed, B cell development proceeds with substitution of the SLC, after a productive VJL recombination, with the light chain (kappa or lambda), that associates with the μ HC and the signaling heterodimer Ig α /Ig β in order to form the BCR [16–19]. Bone marrow development culminates with the formation of immature IgM-expressing B cells, ready to enter the periphery.

Ig β

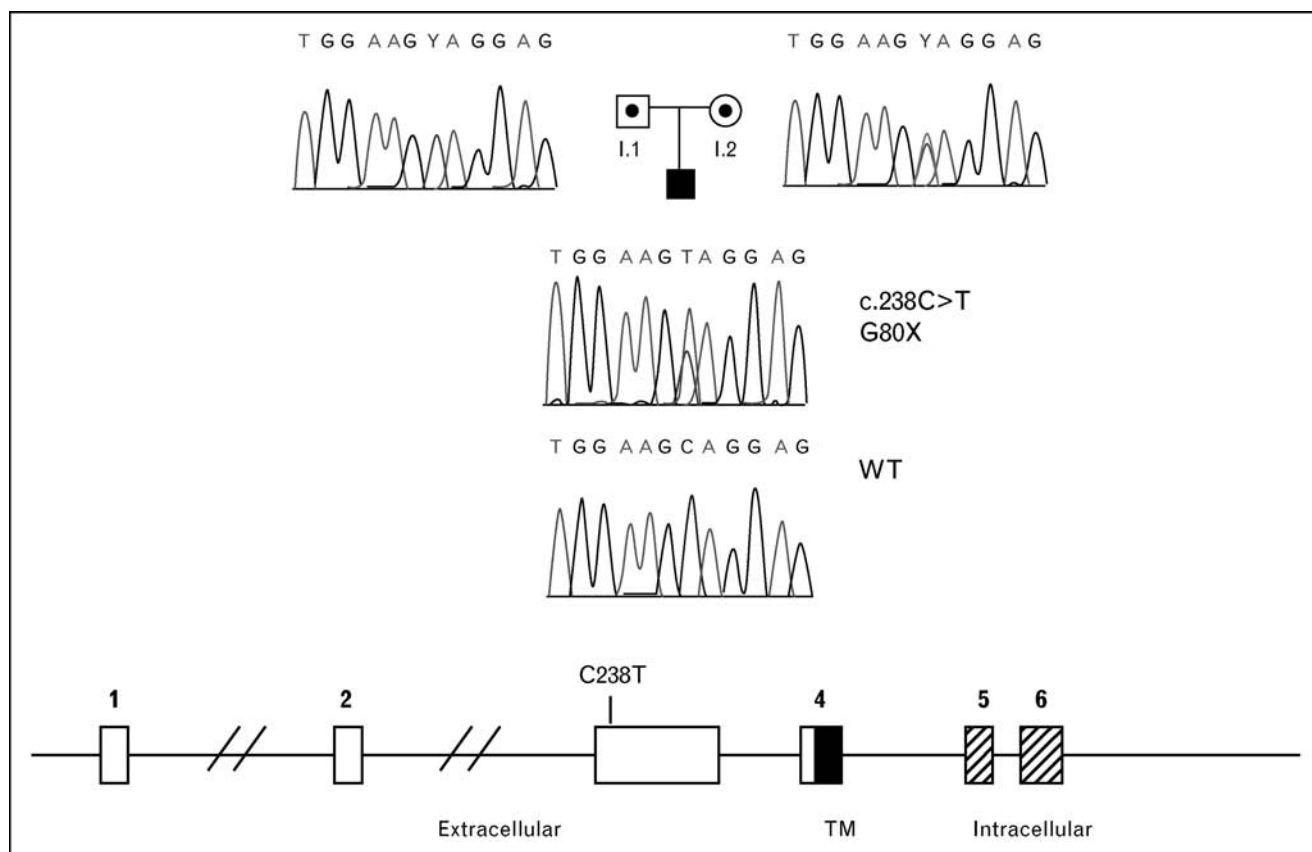
Ig β , a component of the pre-BCR, is encoded by the *B29* gene, localized on chromosome 17. The complete sequence showed immunoglobulin-like structure [20,21]. The important role of Ig β in regulating early B cell development was demonstrated in mice lacking Ig β [22]: the knockout mice had a complete block in B cell development at the immature CD43+B220+ stage. Although immunoglobulin heavy chain diversity and joining segments rearranged normally, variable (V) to DJ recombination and immunoglobulin messenger RNA expression were compromised. Recent investigations regarding its functional significance showed that Ig β presents an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic tail and is therefore proposed to mediate downstream signaling after pre-BCR and BCR

engagement. Ig β is first expressed on the surface of pro-B cells, before the expression of a functional μ HC, associated with calnexin [23]. Although expressed at low levels on pro-B cells, its engagement with an anti-Ig β monoclonal antibody mediated the phosphorylation of downstream tyrosine and serine/threonine kinases, and induced B cell development in the bone marrow of recombination activating gene 2 (RAG-2)-deficient pro-B cells [23]. The essential role of the Ig β molecule in the transition from pro-B to pre-B cell was shown to be Btk-mediated [24]. In pro-B cells, Ig β is not associated with Ig α , exhibits an immature glycosylation pattern, does not associate with SLC, and is retained intracellularly; it appears, however, associated with the Lyn protein tyrosine kinase, confirming a potential functional role [25]. Progression in the pre-B cell stage showed major heterogeneity due to increased glycosylation complexity. Finally, the Ig α /Ig β heterodimer assembles with IgM on the surface of B cell precursors. Besides B cell differentiation in the bone marrow, it was recently demonstrated that Ig β expression is essential for pre-B cell and immature B cell survival [26].

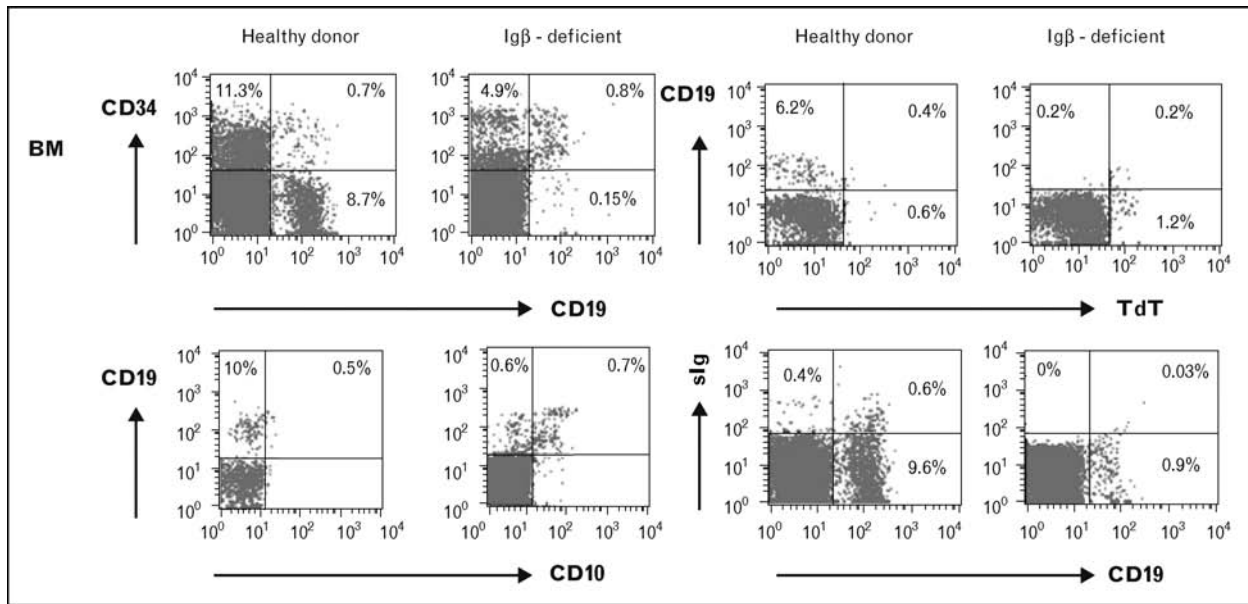
Ig β mutations in humans

Although mutations in components of the pre-BCR such as μ HC, lambda5, and Ig α have already been identified in patients with autosomal recessive agammaglobulinemia, no mutations in Ig β had been described until recently, when two different groups identified two patients with agammaglobulinemia associated with mutations in the *B29* gene encoding the Ig β protein [27[•],28[•]]. The first patient, a 20-year-old Italian male without known consanguinity, presented a homozygous mutation in the Ig β encoding gene [27[•]]. Clinical diagnosis was made at the age of 8 months, when he was admitted for pneumonia and gastrointestinal infection: the immunological work-up showed severe hypogammaglobulinemia in the absence of peripheral B cells (<1%). Replacement therapy was initiated at that point; mutation analysis for Btk was negative. The patient presented a homozygous C>T nucleotide substitution at position c.238 in exon 3 of the *B29* gene, resulting in the replacement of Gln80 with a stop codon within the extracellular domain of Ig β (Fig. 1). The mutation hereby

Figure 1 Mutation analysis of the human *B29* gene



(a) Electropherograms from a portion of exon $\beta 3$ showing the 238 C>T mutation corresponding to amino acid 80 of the immunoglobulin chain, compared with the wild-type sequence of a healthy donor (WT). The sequences of the heterozygous parents (I.1 and I.2) are shown. (b) Schematic representation of the Ig β -encoding gene. Data from Ferrari *et al.* [27[•]].

Figure 2 Bone marrow analysis of the Ig β -deficient patient

Bone marrow mononuclear cells from the Ig β -deficient patient and an age-matched healthy control. The patient showed a marked reduction of CD19⁺ cells and more than 85% of the B-lineage cells were CD34⁺ (upper left). Almost none of the CD19⁺ cells of the patient expressed surface Ig (bottom right). The majority of the CD19⁺ cells of the patient were positive for intracellular TdT staining confirming their pro-B state of differentiation (upper right).

described abrogates the expression of the functional protein and prevents the assembly of the pre-BCR on the cell surface. This was underlined by transfection experiments in *Drosophila melanogaster* Schneider cells: although the wild-type protein was normally expressed within the BCR complex, the mutant Ig β abrogated the expression of the complex. Bone marrow analysis showed a complete arrest at the pro-B cell to pre-B cell stage – a phenotype observed in other known forms of agammaglobulinemia (Fig. 2). Furthermore, human Ig β deficiency closely resembles the phenotype observed in Ig β knockout mice.

The second patient presented a homozygous glycine-to-serine substitution at codon 137 adjacent to the cysteine required for the formation of the disulfide bond between Ig α and Ig β causing a markedly reduced export of the μ HC to the cell surface and resulting in an inability to form disulfide-linked complexes [28^{••}]. This was further demonstrated using retroviral expression vectors that would allow the production of an artificial BCR in 293T cells or Jurkat T cells.

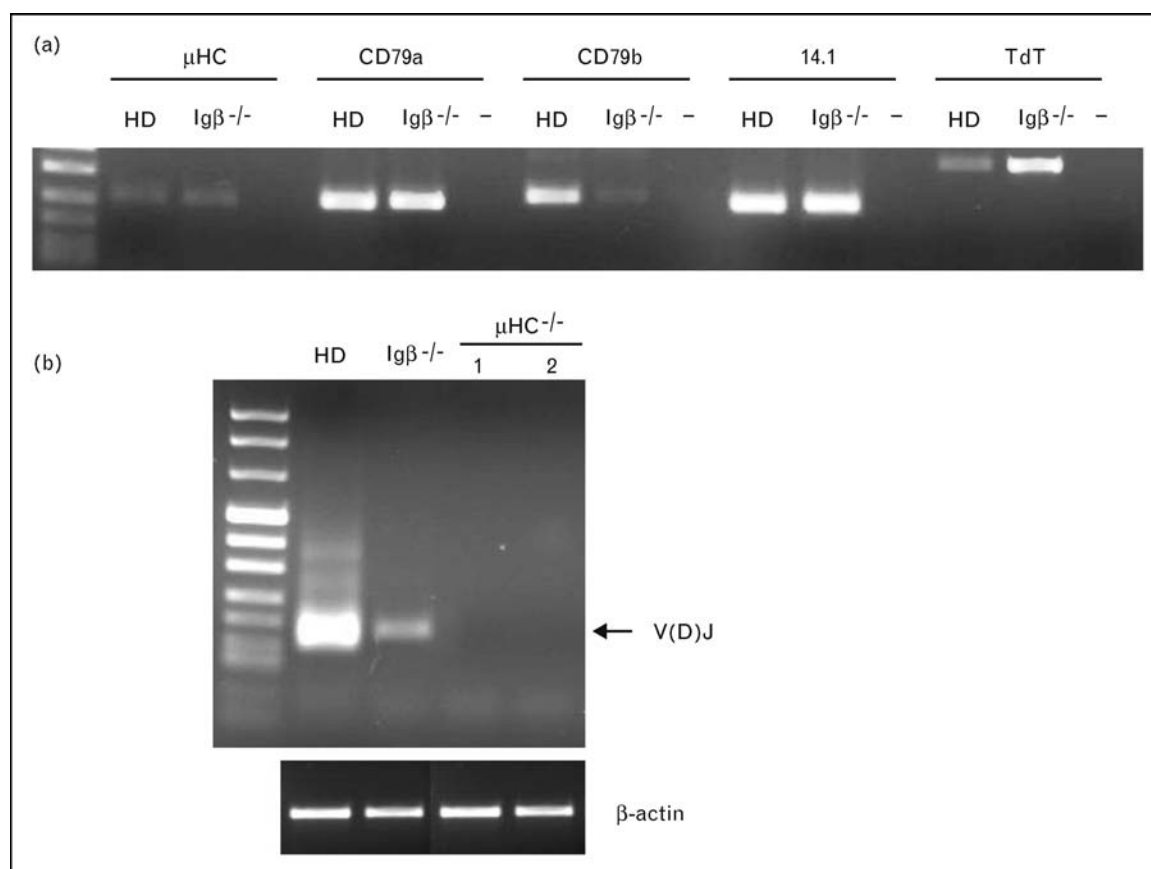
The above mentioned patient had a markedly reduced but still detectable number of CD19⁺ B cells in the peripheral blood. As the pre-BCR is generally expressed at low cell surface density in pre-B cells, the impaired efficiency of expression of the receptor in this patient is likely to fall below the threshold required for the pro-B

cell to pre-B cell transition or for the expansion of the pre-B cell population [28^{••}]. A small number of cells may be able to move through this block.

Gene expression in B cell progenitors

Semiquantitative RT-PCR was performed to evaluate the expression of genes specific to various stages of B cell differentiation. We compared an age-matched control with the Ig β -deficient patient and analyzed transcripts directly linked to early B cell development and immunoglobulin gene expression. β -Actin control amplification showed that equivalent amounts of cDNA were used in each RT-PCR amplification. The TdT transcript associated with the pro-B stage was expressed at the higher level in the patient with Ig β deficiency. This is consistent with the result of the cytofluorimetric analysis of the bone marrow showing that there is an accumulation of TdT-positive cells that did express CD19 (Fig. 2). However, λ 5/14.1, μ HC, and Ig α were expressed in approximately equal amounts in the patient and in the healthy control. The Ig β transcript itself was expressed at lower level probably because the occurrence of a premature stop codon causes nonsense-mediated RNA decay.

In addition we evaluated the amount of V-DJ rearranged μ HC transcripts and we found that they were greatly decreased in the patient with Ig β deficiency as compared to the healthy control. In two patients with μ HC

Figure 3 Semiquantitative RT-PCR analysis of B cell-specific transcripts in the bone marrow

(a) Equal amounts of cDNA from an age-matched healthy control, the patient with $Ig\beta$ deficiency and a cDNA negative template control were amplified using primers specific for μ HC, $Ig\alpha$ (CD79a), $Ig\beta$ (CD79b), $\lambda 5/14.1$, TdT. (b) cDNA samples were analyzed using a primer specific for all VH family members paired with a primer for the CH1 domain of μ HC. β -Actin was used as a control to demonstrate equal concentrations of cDNA.

deficiency the V-DJ rearranged- μ HC transcripts were almost absent (Fig. 3).

When coupled with the immunophenotypic studies, these results further confirm that the block in B cell differentiation in $Ig\beta$ deficiency is at the pro-B to pre-B transition. A similar block at the pre-BCR checkpoint has been described in patients affected by mutations of other members of the pre-BCR, namely μ HC deficiency [5] and $Ig\alpha$ deficiency [29].

Conclusion

The identification of mutations in $Ig\beta$ causing agammaglobulinemia in humans underlines the genetic diversity that characterizes this condition. These novel findings have shed light into the early stages of B cell development in the human bone marrow, adding useful information both from a clinical and a scientific point of view. Considering the underlying monogenetic defect, alternative therapeutic arms may be taken into consideration (i.e. gene therapy). On the contrary, there are still agam-

maglobulinemic patients without a definitive genetic diagnosis; further research is therefore necessary in order to define the underlying molecular and genetic causes underlying this condition.

Acknowledgement

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References and recommended reading

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 592).

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