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Spontaneous mutation in the *Cd79b* gene leads to a block in B lymphocyte development at the C′ (early pre-B) stage

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Abstract

We discovered B lymphocyte deficient mice within a group of B10.A-CD45.1 mice, and established that this deficiency was a recessively inherited trait. Gene mapping and sequence analysis showed a mutation in the 3rd exon of the *Cd79b* gene (c.224G>A) that leads to the generation of a stop codon (W75X) in the mutant mouse. FACS analysis of bone marrow cells showed that the mutant mice did not express the CD79B antigen. In order to establish where the block in development happens, we analyzed $CD43^{pos}B220^{pos}$ B lymphocyte precursors present in the mutant mice, and found that the fraction C′ (corresponding to early pre-B lymphocytes) was absent in the mutant mouse whereas fractions B and C showed a relative accumulation. As expected, we found no IgG or IgA in mutant mice. These results suggest that this CD79b mutant strain may be a useful tool for immunological research into human immunodeficiencies.

Introduction

Primary immunodeficiency manifested by agammaglobulinemia and absence of circulating B lymphocytes is the most common congenital immune deficiency[1]. Since 1993, when the first gene responsible for agammaglobulinemia was identified[2; 3], knowledge of the genome and progress in DNA sequencing have facilitated the discovery of mutations in several other genes in patients with agammaglobulinemias [1]. The most recently reported gene was *CD79B* coding for CD79B antigen (also known as Igβ) with mutations found in 2 patients[4; 5].

Genetically manipulated mice are useful tools for elucidating mechanisms and testing treatment approaches for human diseases. One disadvantage, however, is the need to backcross for many generations to isolate the manipulated gene from other potential operators. Even after many generations, it is difficult to ensure that the observed phenotype is not the result of a linked locus. In the case of agammaglobulinemia, where patients mostly have hypomorphic point mutations, mouse models with similarly restricted defects would be the most ideal for the research.

In this report, we describe a novel mouse with a spontaneous mutation in the *Cd79b* gene leading to a block in B cell development and agammaglobulinemia.

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Conflict of interest
None.

Results & Discussion

While isolating organs from a group of B10.A-CD45.1 mice, we noticed that 2 mice out of 16 did not have Peyer's Patches, and suspected that the absence of B lymphocytes might be the possible cause[6; 7] and analyzed spleens of these mice for B220 positive cells. We found less than 0.5% $B220^{pos}$ cells, confirming that these 2 mice did not have peripheral B cells. Investigating further, we analyzed blood from other mice of the same line from the same vendor and found that 7 out of 30 of those mice did not have blood-borne B cells.

To determine whether this observation was the result of an inheritable mutation, (as opposed to, for example, a B-cell depleting virus), we crossed B cell negative mice to B cell positive mice of the same strain. 100% of the F1 pups had blood-borne B cells, while ~20% (10/51) of the F2 generation were negative, showing a clear recessive inheritance of the trait (Fig. 1a). As a complementation test, we also crossed the B cell negative mice to a strain of B cell negative mice that contained mutation in the Immunoglobulin locus (μMT; mutated in the 3′ end of the mu heavy chain), and found that 100% of the F1 progeny of both crosses contained B cells (not shown). Thus the new mutant was unlikely to be located within the Immunoglobulin locus.

To identify the mutation responsible for the absence of B cells in the mutant mice, we crossed the B cell negative mice to normal mice of strain 129SVE and screened the F2 progeny for the presence of circulating B cells. As expected for a recessively inherited trait, approximately 25% (94/389) of the F2 mice were affected. Using strain-specific genetic markers, we found that the affected locus mapped to a 10 Mb region between rs3723163 and rs13481240 on chromosome 11 (Fig. 1b) that contained approximately 150 genes. Among those lay a gene (*Cd79b*) already known to be involved in B cell development, making this gene the best candidate for further investigation. After direct sequencing of the *Cd79b* gene [8] in the mutant and wild type mice, we indentified a mutation in exon 3 c.224G>A that leads to generation of a stop codon (TGG>TAG; W75X) in the mutant mouse (Figure 1c). The mutation was in the Ig-like domain of CD79b, which is responsible for binding of Igβ to Igα. The two molecules together constitute the signaling part of the B cell antigen-receptor complex, which is expressed throughout B cell development, from early B-cell precursors to plasma cells [9; 10].

As expected, FACS analysis of bone marrow cells showed that the mutant mice we did not express the CD79B antigen, whereas it was strongly expressed on $B220^{pos}$ cells in wild type mice (Figure 1d). We named the mutant mouse strain $B10.Cg\text{-}Ptprc^{a}Cd79b^{m1Ghost}H2^{a}$, in accordance with the Mouse Genome Informatics (MGI) formal nomenclature where Cg states for congenic. For general usage, synonyms B10.A-CD45.1-$Cd79b^{mut}$ or $Cd79b^{mut}$ can be utilized.

A knockout mouse for *Cd79b* has previously been generated and shown to have a complete block in B cell development at the $CD43^{pos}B220^{pos}$ stage[11]. This cell population includes different developmental stages, from pre-proB to early preB cells, that have been designated as fractions A, B, C and C′ [12]. The authors did not find clear differences in the distributions of these fractions between *Cd79b* knockout and wild-type mice. However, because the early preB cells (fraction C′) are the first cells to appear after receiving a signal through IgM/Igβ/Igα complex that stimulates them to enter the cell cycle[12], we surmised that these cells should be missing in the KO mouse (because the pre-BCR complex is not formed). Therefore, we analyzed the proportions of cells undergoing B cell development in the bone marrow of the new mutant, which in principle should resemble the *Cd79b* knockout mouse. Indeed there were several features in common between the new mutant and the *Cd79b* knockout, but also some striking differences. As in the original report on the KO mouse, we found that the B cell development seemed to stop at the $CD43^{pos}B220^{pos}$ stage. However, in contrast to the previous work, we found dramatic changes in the distribution of the cells in fractions A-C. Using CD24

and BP-1 surface staining[13], we found that fraction C′ was virtually absent in the CD79b mutant mouse, whereas fractions B and C showed a relative accumulation (Fig. 2b). Such accumulation would be expected if the mutant B cells were unable to receive a signal via the BCR, as both fractions are immediate precursors of the C′ cells[14]. This result suggests that the Cd79b gene product is necessary for progression into the C′ prime stage, and fits well with current knowledge of the B cell development process, which holds that cells in fraction C′ are large, proliferating pre-B cells that have recently received a functional signal via the pre-BCR complex[15].

There are two potential reasons why we obtained different results from those previously published on the *Cd79b* KO mouse. The first is that C and C′ populations in B6 mice have been difficult to discriminate by FACS (personal communication from R. Hardy). The last decade has seen a great deal of technological improvement in flow-cytometry that allowed us to overcome this problem. The second reason is related to the methods by which the mutations were generated. Our mutant mouse is a spontaneous mutant that has only a single point mutation, the remainder of its genome coming from the inbred line. This provides a unique opportunity to estimate precisely where the block in development occurs, without the effect of any other potential confounding factors that may exist in a knock-out mouse, where it is difficult to rule out the effect of other genes that may not have been removed during the backcrossing process.

Some investigators have found low levels of IgG, IgA in μMT B cell knockout (BcKO) mice[16], which have a block in the development of B cells at a stage similar to our mutant. Others, however, have not[17], and human patients with similar mutations lack IgA[18]. We therefore measured levels of total IgA and IgG in the blood of *Cd79b* mutant, in control heterozygous mice, and in μMT BcKO mice and their corresponding controls. We found neither IgA nor IgG in the *Cd79b* mutant or the μMT BcKO mice (Figure 3). Although it is still not clear why our results in the μMT mice differ from those of Macpherson et al, one hypothesis is that it relates to differences in the flora of the different mouse colonies. In addition, Hasan et al have shown that there is additional locus not linked to immunoglobulin locus which is critical for differences in immunoglobulin production between μMT BcKO mice on BALB/c and B6 backgrounds. The result regarding the absence of immunoglobulins in *Cd79b* mutant is quite straightforward and is in agreement with aglobulinemias in *Cd79b* mutant patients[4; 5].

Different mutations in the *Cd79b* gene can lead to different outcomes. For example, leukemia has been reported in human patients with mutations in *Cd79b* gene that lead to changes in the protein but not to its complete or significant ablation[19]. Notably, the mutation found in the new mouse reported here is remarkably similar to the mutations found in two immunodeficient patients that had mutations in the *Cd79b* gene. Indeed, both patients had mutations leading to a stop codon in the 3rd exon of the *Cd79b* gene, and had no or extremely low numbers of B cells and antibodies[4; 5]. These results suggest that this new mutant strain could be a useful tool for immunological research into the human immunodeficiencies, as it closely resembles the phenotype of the immunodeficient human patients; it has a homogeneous inbred genome, a congenic marker, and a wild type strain that differs only by this single mutation.

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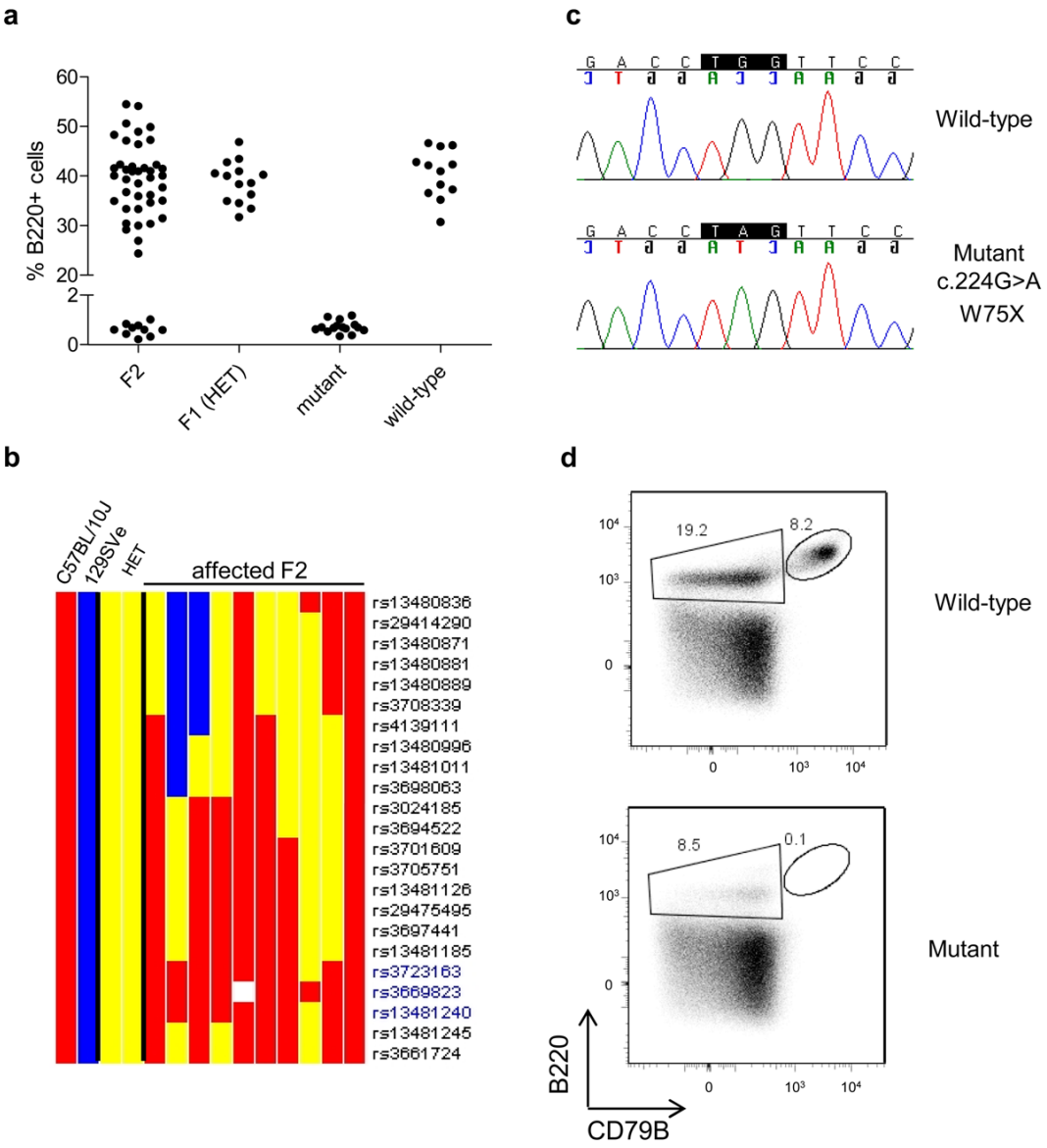

Figure 1. Finding the genetic defect in the spontaneous mutant mouse

a) Percentage of $B220^{pos}$ positive cells in blood of wild type, mutant, F1 heterozygous (HET), and F2 mice.

b) Chromosome 11 map for SNP markers in B10 (red), 129SVE (Blue), HET – heterozygous (yellow), and F2 affected (absence of B220+ cells) mice. Technical failure to detect a SNP shown in white. The region between rs3723163 and rs13481240 was the only place in the genome where all of the affected F2 mice had SNPs inherited from the affected B10.A grandparent (red).

c) Chromatograms of the relevant portion of exon 3 of the *Cd79b* gene from the mutant and wild type mice.

d) Surface expression of the B220 and CD79B antigens on bone marrow cells of the mutant and wild type mice (gated on 7AAD negative cells).

Mice & Gene Mapping: Mice carrying the $Cd79b^{W75X}$ mutation were bred in the Taconic Farms NIAID contract facility. For gene mapping studies, (mutant x 129/SvEv)F_1 mice were intercrossed to generate F_2 progeny, which were screened for presence of B cells in peripheral

blood. DNA samples from 10 affected F_2 mice, two F_1 mice, one 129SVE and one B10.A mouse (the background strain where the mutant mice were found) mouse were genotyped for strain-specific SNPs using the SNP array from Illumina (mouse low density linkage panel) by the Partners HealthCare Center for Personalized Genetic Medicine (PCPGM), Harvard Medical School. *Cd79b* gene and the promoter region were sequenced in the mutant (2 pool samples, each pool containing DNA samples from 2 individual mice) and B10.A-CD45.1 mice (one DNA pool sample containing samples from 5 individual mice). Experiments were approved by the NIAID Animal Care and Use Committee at NIH, which is accredited by the AALAC. The mutant mouse B10.Cg-Ptprc^{a}Cd79b^{m1Ghost}H2^{a} is registered in the MGI database under accession number 3829352.

FACS staining: Proteins expressed on the surface of blood and bone marrow cells were stained following standard protocols. The fluorochrome-conjugated antibodies were B220-PE (clone RA3-6B2; BD Pharmigen, San Jose, CA, USA), anti-CD79B-FITC (clone HM79b; BD Pharmigen, San Jose, CA, USA); 7-Aminoactinomycin D (7AAD; BD) was used to identify living cells. Data were acquired on a BD FACSCanto™ flow cytometer and analyzed using FlowJo software.

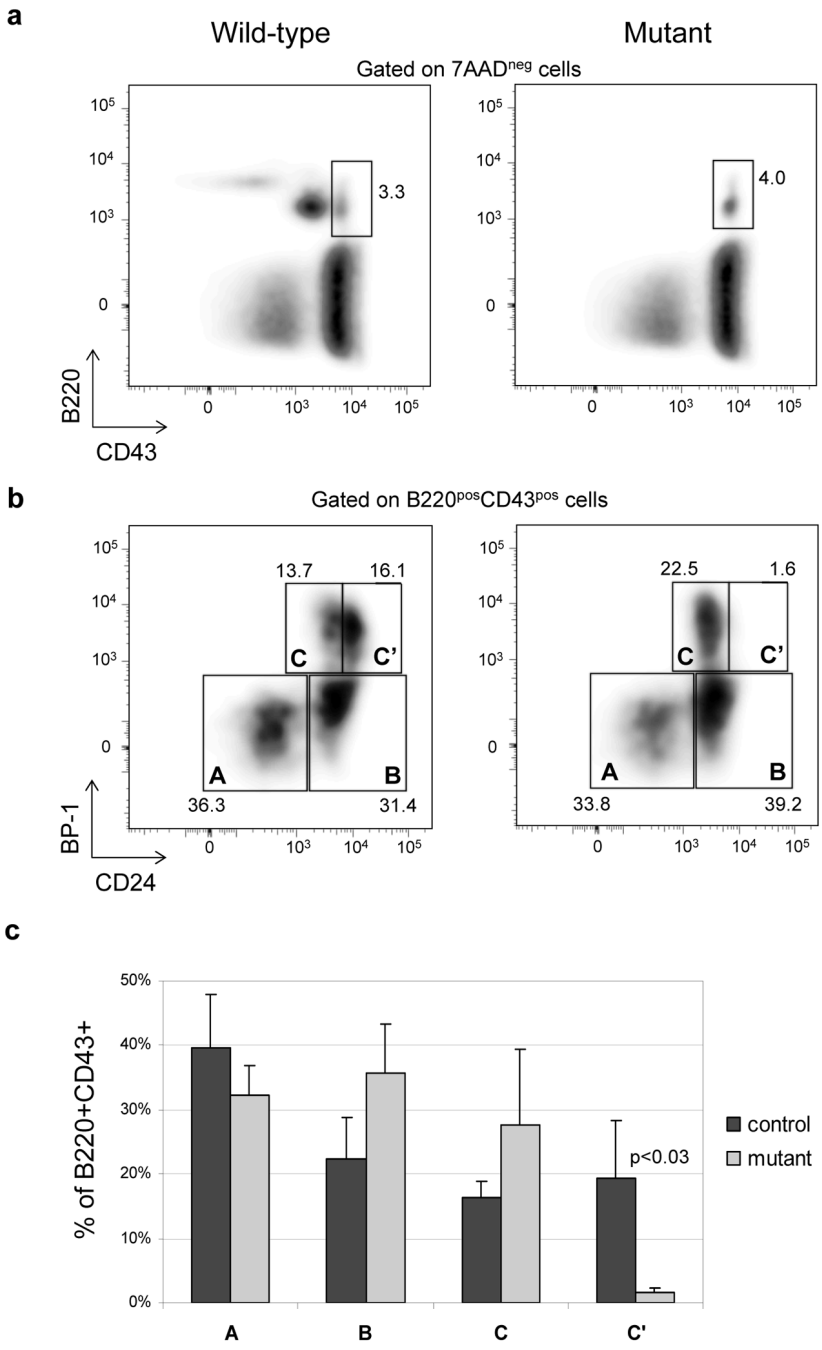

Figure 2. Phenotype of the bone marrow cells from Cd79b mutant and wild mice for pro-B to pre-B transition markers

a) Surface expression of B220 and CD43 on bone marrow cells of mutant and wild type mouse.
b) A, B, C, C′ fractions in bone marrow cells of mutant and wild type mouse defined by surface expression of BP-1 and CD24.
c) Percentages (mean, standard deviation) of A, B, C, C′ fractions of in bone marrow cells of mutant and wild type (n=4 in each group). Control mice consisted of 2 wild-type and 2 heterozygous animals.

FACS staining: All procedures were performed as described in Figure 1. Following fluorochrome-conjugated antibodies were used for staining: anti-B220-APC-Alexa750 (clone

RA3–6B2, eBioscience, San Diego, CA, USA), anti-CD43-APC (BioLegend, San Diego, CA, USA, clone 1B11), anti-CD24-FITC (BD Pharmigen, San Jose, CA, USA, clone M1/69), anti-BP1-PE (BD, clone BP-1). Bone marrow from 2–4 months old female mice was used for staining.

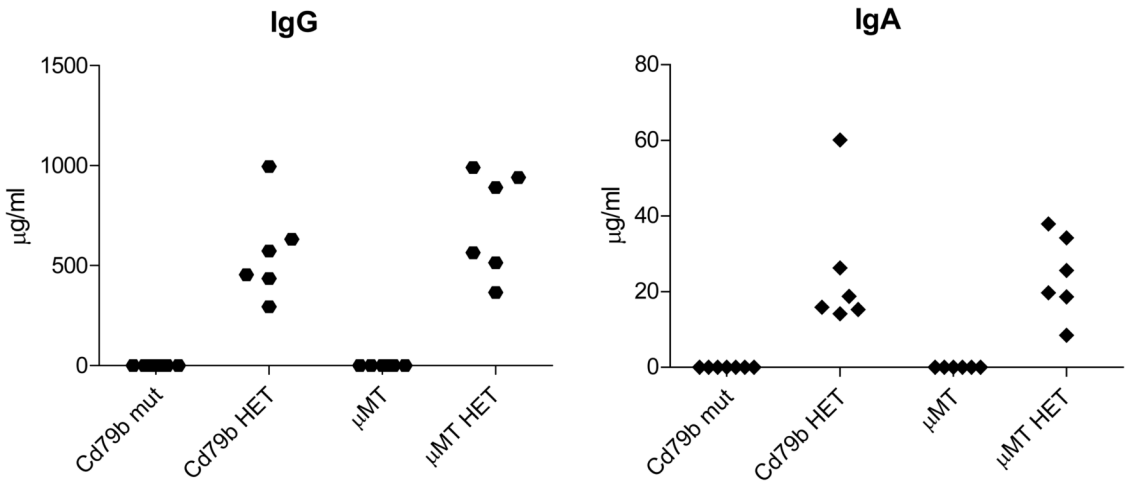

Figure 3. Serum levels of IgG and IgA in CD79b mutant (mut), µMT mice and their corresponding heterozygous (HET) littermates

Immunoglobulin levels were measured in serum samples using ELISA kits from Immunology Consultants Lab Inc (Newberg, OR, USA). Mice were females, 3–6 months of age, the background strain for all mice was B10.A. The original mutant and wild-type mice were obtained from the NIAID contract facility at Taconic Farms, and bred to N2 generation to obtain mutant and heterozygous littermates.