

Letter to the Editor

Early and late B-cell developmental impairment in nuclear factor kappa B, subunit 1–mutated common variable immunodeficiency disease

To the Editor:

The nuclear factor kappa B (NF- κ B) signaling pathway plays an important role in immune cell biology.¹ Both the classical (nuclear factor kappa B, subunit 1 [NF- κ B1]; p105/p50) and the alternative (nuclear factor kappa B, subunit 2 [NF- κ B2]; p100/p52) NF- κ B pathways have been largely studied mainly in animal models.¹ Regarding B cells, the role of NF- κ B1 was underlined in a murine nf- κ B1 knockout model in which peripheral B cells showed defective maturation, defective isotype switching, and impaired humoral immune responses.^{1–3} A similar, although more pronounced, immunologic phenotype was observed in the nf- κ B2 knockout mice, with defective secondary lymphoid organ development and impaired B-cell development both in early (bone marrow) and in late (periphery) stages with defective humoral responses both to T-dependent and to T-independent antigens.¹ The role of NF- κ B2 in human B-cell development was recently defined in patients carrying monoallelic mutations in NF- κ B2, leading to common variable immunodeficiency (CVID)-like disease with autoimmunity and defects in late stages of peripheral B-cell maturation.^{4,5} Monoallelic mutations in NF- κ B1 leading to p50 haploinsufficiency were recently described in a limited number of patients with CVID⁶; however, data regarding the effect of monoallelic NF- κ B1 mutations on B-cell development are scarce. We report on 2 patients carrying monoallelic mutations in NF- κ B1, one of which was novel, and describe novel findings on the effect of these mutations on B-cell development both in early (bone marrow) and in late (periphery) stages of maturation.

Patient 1 was born to Italian nonrelated parents. He came to our attention at the age of 7 years due to recurrent respiratory infections. His immunological workup showed normal differential white blood cell count with low immunoglobulin serum concentrations and absent responses to recall vaccinations; frequency of peripheral B cells was low (CD19⁺ B cells, 3.2% of total lymphocytes), whereas that of T cells was normal (Table I). The patient was put on immunoglobulin replacement treatment with resolution of the infectious episodes. During follow-up, he developed autoimmune thyroiditis and gastrointestinal symptoms that led to the initial diagnosis of celiac disease. Gluten-free diet did not resolve the villous atrophy and diagnosis of autoimmune enteropathy was then made. Treatment with oral budesonide led to complete clinical and histological remission. Patient 1 has developed a gastric adenoma that is under regular endoscopic follow-up. Patient 2 has been previously reported⁶ (Table I).

Genetic analysis of 98 known and putative genes by targeted next-generation sequencing revealed the presence of the novel c.1517delC monoallelic frameshift mutation in the *NFKB1* gene in patient 1 (Fig 1, A). No additional putative disease-causing mutations were evident. The mutation predicts a premature termination of translation (p.Ala506Valfs*17) and thus expression of an aberrant p50 but a lack of expression of the p105 precursor from the mutant allele. Patient 2 harbors the

TABLE I. Clinical and immunologic features of the index patients harboring monoallelic *NFKB1* mutations

Features	Patient 1	Patient 2*
Sex	Male	Female
Age at diagnosis (y)	7	30
Current age (y)	40	55
IgG (mg/dL) [†]	160	181
IgA (mg/dL) [†]	14	6
IgM (mg/dL) [†]	14	48
Recall response to vaccinations		
Tetanus toxoid	Absent	NA
Hepatitis B	Absent	NA
Lymphocyte subsets [†]		
CD3 (%)	83.984.3	
CD4 (%)	39.758	
CD8 (%)	53.523.2	
CD19 (%)	3.210.5	
CD56 (%)	10.64.7	
Proliferative response to mitogens		
Anti-CD3, anti-CD3 + IL-2, PMA + I, PHA	Normal	NA
Respiratory infections	Pneumonia, sinusitis	Pneumonia, recurrent sinusitis
Bronchiectasis	—	—
Autoimmunity	Autoimmune thyroiditis, autoimmune enteropathy	—
Other	Gastric adenoma	—
<i>NFKB1</i> mutation	c.730+4A>G p.Asp191_ Lys244delinsGlu p50 haploinsufficiency	c.1517delC p.Ala506Valfs*17 premature termination

NA, Not available; PMA + I, phorbol 12-myristate 13-acetate and ionomycin.

*Patient 2 was previously reported in Fliegauf et al⁶ (patient NL1-36).

[†]At diagnosis.

previously reported c.730+4A>G monoallelic mutation in the *NFKB1* gene, which leads to in-frame skipping of exon 8 (p.Asp191_Lys244delinsGlu), rapid decay of the mutant p105 Δ Ex8 precursor, and thus to p50 haploinsufficiency.⁶ Considering the limited available data in the literature, we decided to evaluate the B-cell compartment in the index patients. Early B-cell development in the bone marrow was evaluated for patient 1. In detail, early stages of B-cell differentiation appeared impaired, with a partial maturational arrest at the pre-BI stage, suggesting therefore a direct role for NF- κ B1 in the early stages of human B-cell development (Fig 1, B, lower panel; see Fig E1 in this article's Online Repository at www.jacionline.org). Peripheral B-cell evaluation was performed for both patients and was compared with a healthy donor (HD) and a previously reported patient⁵ harboring a *de novo* c.2557C>T monoallelic mutation in *NFKB2* (patient 3). The mutation in NF- κ B2 leads to a premature termination of translation (p.Arg853*) and causes a functional haploinsufficiency due to expression of a nonprocessable p100 precursor. Patient 3 was included to better define the specific role of the classical and the alternative NF- κ B pathways in peripheral B-cell maturation. Patient 1 showed an almost exclusive peripheral CD21^{lo} pattern (CD21^{lo}: 91.1% [23 cells/ μ L] of peripheral B cells

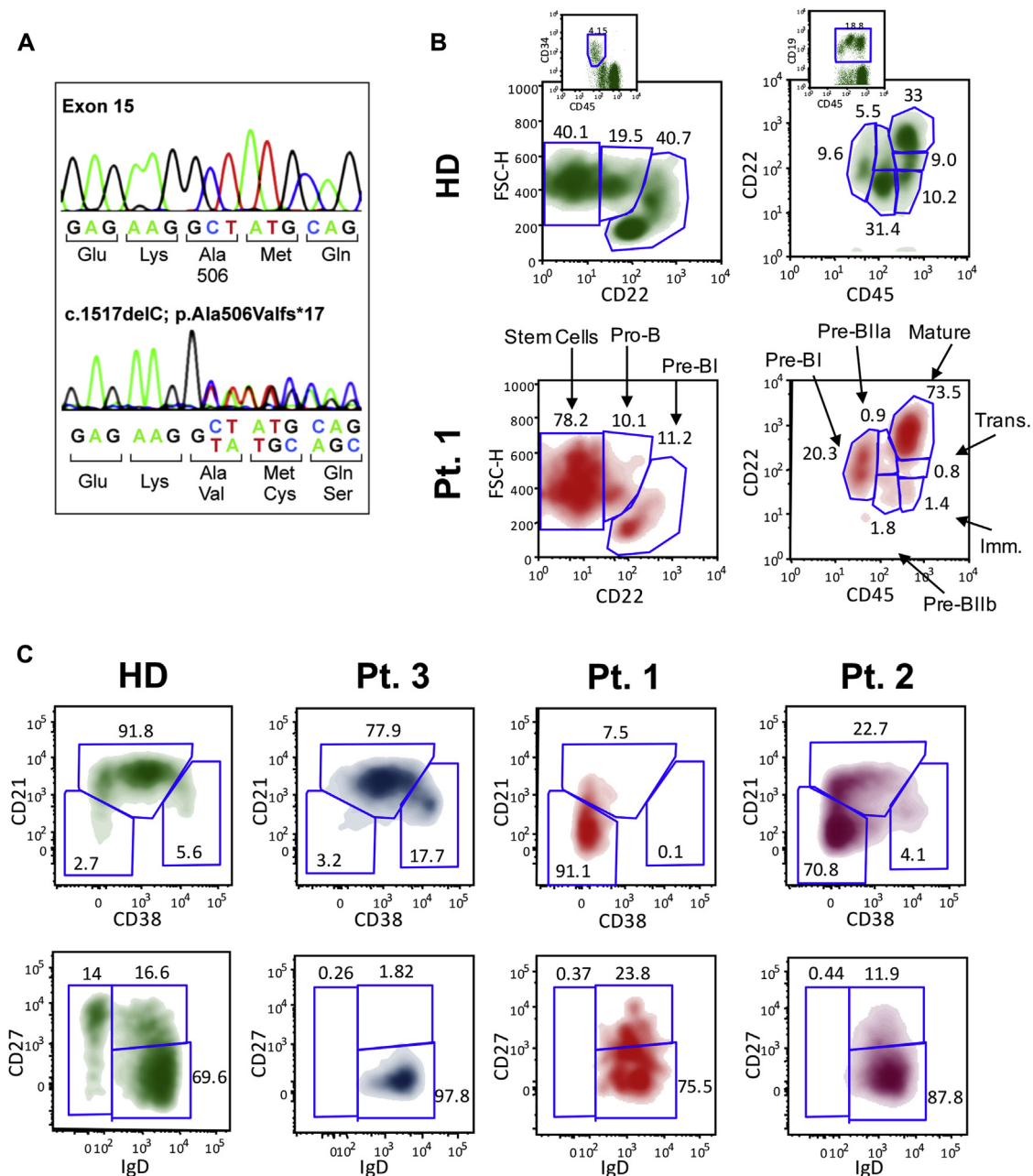


FIG 1. NF- κ B1 mutations and human B-cell development. **A**, Electropherogram showing the novel monoallelic mutation in *NFKB1* from the index patient 1. **B**, Flow cytometric evaluation of early B-cell development in the bone marrow from an age-matched healthy donor (HD; green) and the *NFKB1*-mutated patient (Pt 1; red). The left plot shows the cell subsets upon gating on the lymphocyte subset CD34⁺CD45⁺ (small upper left plot), whereas the right dot plot shows the cell subsets upon gating on the CD19⁺CD45⁺ B-cell subset (small upper right plot). **C**, Flow cytometric evaluation of peripheral CD19⁺ B cells from an HD (green) and patients 1 (Pt 1; red), 2 (Pt 2; purple), and 3 (Pt 3; blue). Imm., Immature; trans., transitional.

[27 cells/ μ L]) (Fig 1, C, upper panel). A similar expansion of CD21^{lo} B cells was observed in patient 2 (CD21^{lo}: 70.8% [120 cells/ μ L] of peripheral B cells [190 cells/ μ L]). Interestingly, this expansion of CD21^{lo} B cells was not observed in the NF- κ B2-mutated patient (CD21^{lo}: 3.2% [1 cell/ μ L] of peripheral B cells [41 cells/ μ L]), suggesting therefore an intrinsic role of the classical NF- κ B pathway in the biology of CD21^{lo} B cells in CVID. Based on the data presented here, the expansion of the CD21^{lo} population that was reported in a limited number of

patients with CVID without a genetic diagnosis⁷ may be related to mutations in *NFKB1* and to alterations in the classical NF- κ B pathway in general. Furthermore, the expansion of the CD21^{lo} population has been associated with systemic lupus erythematosus and autoimmune phenomena,⁸ as observed in patient 1. Both patient 1 and patient 2 showed a complete absence of CD27⁺ switched memory B cells: 0.37% (patient 1) and 0.44% (patient 2) on mature CD21^{hi}CD38^{lo/dim} cells versus 14% (HD) (Fig 1, C, lower panel), underlining an important role for

NF- κ B1 in the late stages of human peripheral B-cell maturation. Patient 3, carrying the NF- κ B2 mutation, completely lacked both CD27⁺IgD⁺IgM⁺ unswitched (patient 3: 1.82% vs HD: 16.6%) and switched memory B cells (patient 3: 0.26% vs HD: 14%), thus suggesting an important role for both the classical and alternative NF- κ B pathways in human peripheral B-cell maturation (Fig 1, C, lower panel). All patients lacked CD38⁺⁺CD27⁺⁺ B cells, as typically observed in CVID (data not shown).

Taken together, our data provide evidence for a novel role for NF- κ B1 in human B-cell development, both in the bone marrow and in the periphery. Mutations in NF- κ B1 affect early stages of human B-cell development. Data from animal models suggest an intrinsic role for nf- κ b1 in immature bone marrow B cells,⁹ thus strengthening our findings. The B-cell receptor signaling, essential for early stages of B-cell development, depends on the classical NF- κ B pathway¹⁰ and therefore NF- κ B1 haploinsufficiency could affect this cascade. B-cell memory development is impaired in the presence of both *NFKB1* and *NFKB2* monoallelic mutations, although the latter appears to act at an earlier stage of peripheral B-cell maturation. This peripheral maturational defect may depend on impairment of the B-cell receptor and B cell-activating factor receptor signaling cascades, respectively, dependent on the classical (NF- κ B1) and alternative (NF- κ B2) NF- κ B pathways. Furthermore, the CD21^{lo} population appears particularly expanded in the presence of monoallelic *NFKB1* but not *NFKB2* mutations, suggesting an intrinsic role for the classical NF- κ B pathway in the biology of the CD21^{lo} B-cell population, opening novel possibilities for better understanding this peculiar B-cell subset, both in CVID and in autoimmune diseases, such as systemic lupus erythematosus.

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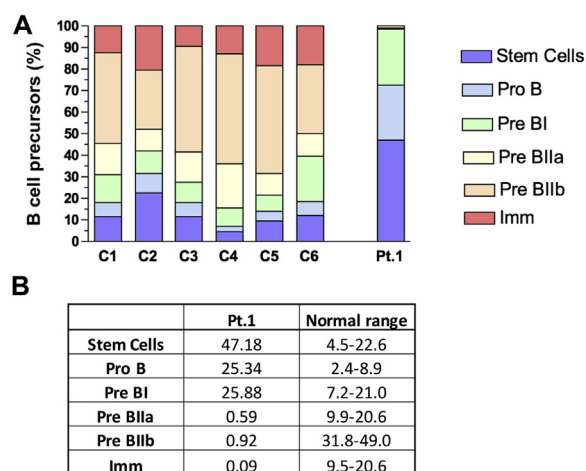


FIG E1. Bone marrow evaluation of B-cell development was done by multicolor flow cytometry in the NF- κ B1-mutated patient and 6 age-matched healthy controls. **A**, Bar graph showing the distribution of the different B-cell subsets during bone marrow B-cell development. **B**, Detailed percentage description of the different B-cell subsets in the bone marrow comparing the NF- κ B1-mutated patient and 6 age-matched healthy controls. *Imm*, Immature; *Pt. 1*, patient 1.