Agnammaglobulinemia with normal B-cell numbers in a patient lacking Bob1

To the Editor:

We describe a 3-year-old boy (P-FR1) from consanguineous white parents lacking immunoglobulins despite normal total B-cell numbers. Healthy until the age of 18 months, he developed recurrent respiratory infections, followed by a progressive central nervous system disease with progressive spastic tetraparesis from 24 months onward. Cerebral magnetic resonance imaging revealed enlarged outer cerebral spinal fluid spaces and hypomyelination of the white matter. A chronic viral disease was suspected. Cerebral spinal fluid analysis showed moderate pleocytosis but negative microbial PCR results (for further clinical details, see the Methods section of the Online Repository at www.jacionline.org). A biopsy for brain tissue PCRs was declined by the parents. P-FR1’s brother died of Zellweger syndrome caused by a homozygous mutation in PEX16 (p.His231Arg) at the age of 14 years. P-FR1 and his sister were heterozygous for this mutation (Fig 1, A) and did not present clinical or laboratory findings of Zellweger syndrome. Exome sequencing performed on P-FR1 and his sister (see Tables E1 and E2 in the Online Repository at www.jacionline.org) revealed the frameshift mutation c.233delC in POU2AF1 (p.Thr78Lysfs*63 [see Fig E1, A in the Online Repository at www.jacionline.org]) that was homozygous in P-FR1 and heterozygous in his parents and sister (Fig 1, A). POU2AF1 encodes Bob1 (also called OBF-1 or OCA-B). Although POU2AF1 transcripts were detected (Fig 1, B), Bob1 protein was absent in a P-FR1 EBV B-cell line (Fig 1, C and see Fig E1, B) and in HEK293T cells transfected with the mutated Bob1 compared with the wild-type Bob1 control (see Fig E1, C), indicating instability of the aberrant fusion protein. In B cells, Bob1 is a transcriptional coactivator that confers octamer-dependent specificity to the transcription factors Oct-1 and Oct-2.1 P-FR1 had normal B-cell numbers but disturbed B-cell differentiation, with decreased class-switched memory cells and a relative increase in atypical-memory cells (IgG+ or IgA+ CD27+) [Fig 1, D and E]. His T cells were normal in terms of distribution and activation (Fig 1, D and see Fig E1, D and E) except for a reduction in circulating T-follicular helper (Tfh) cells (Fig 1, D and E).

P-FR1–naive B cells showed an abnormal expression pattern of surface receptors and signaling molecules, with low levels of IgD, IgM, CD79α, CD79β, and Syk (Fig 2, A and see Fig E1, F). Indeed, Bob1 can directly regulate expression of B-cell receptor (BCR) signaling molecules by interacting with octamer motifs in their promoter regions, as described for CD79β.2 Low CD79β may contribute to low BCR and CD79α expression, as shown in B-cell lines.3 Additionally, interaction with Bob1 has been reported to control the stability of Syk.4 Also, BAFF-R expression and CD22 expression were reduced in P-FR1 B cells. The abnormal pattern of surface expression was confirmed in a P-FR1–derived EBV B-cell line (see Fig E2, A in the Online Repository at www.jacionline.org). Their lentiviral reconstitution with wild-type Bob1 control (see Fig E1, C) showed some immunologic manifestations of P-FR1 showed some overlap with the Bob1 knockout (Bob1−/−) mice, including dysregulated surface expression of BCR, Syk, CD79β, and BAFF-R,5,6 defect in B-cell activation,6 and impaired in vivo T-dependent responses, with a lack of germinal centers and strongly reduced IgG.1 However, some significant
differences were also observed. Bob1−/− mice showed a 2-fold reduction in B-cell numbers, which were normal in the patient. Although serum IgM antibodies were absent in P-FR1, IgM levels were only slightly reduced in Bob1−/− mice, which was attributed to their unaffected B1 B-cell compartment, the existence of which is debated in humans. Furthermore, expression of CD22, which negatively regulates BCR activation, was increased in Bob1−/− mice and low in P-FR1.7 This may have resulted from the different source of the B cells examined, the spleen in Bob1−/− mice, and the blood in P-FR1. Interestingly, stimulation of Bob1−/− murine B cells with LPS resulted in plasmablast formation and immunoglobulin secretion in vitro.8 TLR4 is not expressed in human B cells, but stimulation with TLR9 agonist in P-FR1 was not able to restore plasmablast formation.

This is the first report of a patient with a Bob1 deficiency caused by a homozygous POU2AF1 null mutation resulting in a severe B-cell–intrinsic defect. The paucity in Tfh cells may derive from dysregulated Bcl6 and/or from an altered B-cell–T-cell interaction that is essential in the first phase of Tfh cell differentiation. Bob1-deficient B cells respond poorly to activating signals. BCR signaling was reduced and plasmablast development and immunoglobulin secretion seemed abrogated in the patient. These functional impairments
are Bob1-dependent because reconstitution experiments restored plasmablast development, immunoglobulin secretion, dysregulated surface expression of BCR complex molecules and costimulatory and survival signaling molecules. Overall, these data point to a role for Bob1 in the late stages of human B-cell development, regulating not only activation but also survival, possibly by controlling the expression of key mediators such as BAFF-R and CD79β. In addition, Bob1 deficiency represents a unique example of agammaglobulinemia with normal B-cell numbers. As Bob1 downregulation has been found in non–immunoglobulin-secreting Hodgkin lymphoma, a careful clinical follow-up for the development of lymphoma should be performed in P-FR1. Whether the neurodegenerative symptoms are the consequence of an infection, as is frequently observed in Bruton agammaglobulinemia, or related to an additional genetic disorder remains to be determined, also because central nervous system involvement was not reported in Bob1−/− mice.

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**FIG 2.** Bob1 mutation results in intrinsic B-cell defects. A, Phenotype of naive CD19+CD27−IgD+ B cells from P-FR1 and 2 healthy donors (HDs). B and C, Fold changes of the median fluorescent intensity (MFI) of phosphoproteins following stimulation with anti-IgM (5 minutes [B]) or CD40L (15 minutes [C]) compared with unstimulated (US) naive CD19+CD27−IgD+ B cells of P-FR1 and 2 HDs. D, Naive CD19+CD27− B cells from P-FR1 and HDs were stimulated with CD40L+IL-21 and monitored by flow cytometry. The envelope graph shows means ± ranges of data. E, IgM and IgG concentrations in the supernatant of culture by ELISA. F, Lentiviral reconstitution of CD40L+IL-21–stimulated naive CD19+CD27− B cells from P-FR1 with the human wild-type sequence of Bob1 (Lenti-Bob1) or green fluorescent protein (GFP)-expressing control vector (Lenti-GFP). Plasmablast development, defined as CD38high-expressing cells, among GFP+ cells at day 9. G, IgM concentration in the supernatant of the reconstitution experiment was quantified by ELISA. A–C, One experiment with P-FR1 and 2 age-matched HDs. D, Two experiments with P-FR1 and 3 age-matched HDs, analyzed in at least duplicates. E, Symbols represent replicates from the activation experiments. F, One experiment analyzed in at least duplicates. G, Symbols represent technical replicates from the reconstitution experiment. FMO, Fluorescent minus 1.
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REFERENCES