B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans

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B-cell survival depends on signals induced by B-cell activating factor (BAFF) binding to its receptor (BAFF-R). In mice, mutations in BAFF or BAFF-R cause B-cell lymphopenia and antibody deficiency. Analyzing BAFF-R expression and BAFF-binding to B cells in common variable immunodeficiency (CVID) patients, we identified two siblings carrying a homozygous deletion in the BAFF-R gene. Removing most of the BAFF-R transmembrane part, the deletion precludes BAFF-R expression. Without BAFF-R, B-cell development is arrested at the stage of transitional B cells and the numbers of all subsequent B-cell stages are severely reduced. Both siblings have lower IgG and IgM serum levels but, unlike most CVID patients, normal IgA concentrations. They also did not mount a T-independent immune response against pneumococcal cell wall polysaccharides but only one BAFF-R-deficient sibling developed recurrent infections. Therefore, deletion of the BAFF-R gene in humans causes a characteristic immunological phenotype but it does not necessarily lead to a clinically manifest immunodeficiency.

B lymphopenia | primary immunodeficiency | recessive mutation

B-lymphocyte survival is maintained by tonic signaling of the B-cell antigen receptor complex (1) and by signals induced after binding of the cytokine BAFF/BLYS to the BAFF-receptor (BAFF-R), a member of the TNF receptor superfamily (2). In humans, BAFF-R is encoded by three exons of the *TNFRSF13C* gene located on chromosome 22q13. Its transcript is translated into a type III transmembrane protein of 184 aa residues expressed by all surface Ig^+ B cells but not by plasma cells (3). BAFF, the only ligand of BAFF-R, is secreted by cells of nonhematopoietic as well as of hematopoietic origin, including monocytes, macrophages, neutrophils, and activated B cells (2, 4).

In BAFF-R–deficient mice, B cells develop normally up to the stage of IgM^+ immature/transitional B cells but cannot complete maturation in the spleen, as BAFF/BAFF-R–dependent survival signals are missing (5–8). Therefore, the numbers of follicular and marginal zone but not of transitional B cells are reduced by more than 95% (6). About 20% of B cells passing the developmental block mount only weak high-affinity antibody responses against T-independent and T-dependent antigens (6, 7). Because, in the gut of BAFF-R–deficient mice, mucosal IgA-secreting plasma cells develop normally, they seem not to require BAFF-R signals (6)

Common variable immunodeficiency (CVID) includes many heterogeneous syndromes of unknown origin characterized by hypogammaglobulinemia and recurrent respiratory infections (9). Most patients have normal numbers of T and B cells, but approximately 10% of CVID patients are B-lymphopenic (10, 11). Although this group may include a few cases of BTK deficiency (12), most B-lymphopenic patients have unknown defects, some of which may affect genes regulating early B-cell development and/or B-cell survival (13). Searching for genetic defects affecting B-cell homeostasis, we identified two related individuals carrying the same homozygous deletion within the *TNFRSF13C* gene removing part of the BAFF-R transmembrane region. Human BAFF-R deficiency strongly impairs the development and homeostasis of follicular, IgM memory/marginal zone, and class-switched memory B cells. However, in contrast to the murine BAFF and BAFF-R mutants, the human deficiency shows a late onset and variable penetrance, as it does not inevitably lead to a clinically overt immunodeficiency.

Results

Identification of Human BAFF-R Deficiency. Searching for genetic defects affecting B-cell homeostasis, we screened a cohort of 138 CVID patients for individuals with low numbers of peripheral B cells. We found 35 patients (25%) with less than 5% B cells and 10% ($n = 14$) with less than 3% B cells. BAFF serum levels were analyzed in 14 patients with less than 3% B cells, BAFF-R surface expression in 40 additional patients and BAFF binding to B cells in 18 additional patients, respectively. Similar to a previous report (14), BAFF concentrations were significantly higher in sera of CVID patients than in controls (Fig. 1*A*). For BAFF-R expression we found weaker signals in eight patients including P1, who showed the lowest BAFF-R signal (Fig. 1 *B* and *C*). In whole-cell lysates of Epstein-Barr virus (EBV)–immortalized B cells from P1 BAFF-R protein expression was also undetectable by Western blot analysis (Fig. 1*D*), and RT-PCR of total RNA from P1 PBL revealed a 20–30-bp shorter *TNFRSF13C*-specific fragment than RNA isolated from healthy donor PBL (Fig. 1*D*). Because these results suggested that P1 might carry a BAFF-R mutation, we sequenced the *BAFF-R* gene of P1 and of all the patient's family members (Fig. 2*A*) and detected a homozygous 24-bp in-frame deletion (del89–96),

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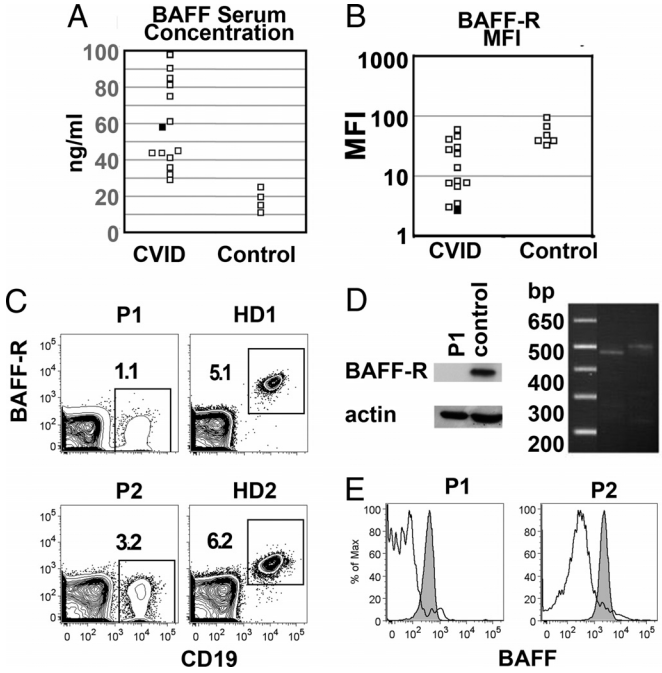

Fig. 1. Identification of BAFF-R-deficiency. (*A*) BAFF serum levels (ng/ml) in CVID patients and controls. Filled square, P1. (*B*) Mean fluorescence intensity of BAFF-R signals detected by flow cytometry of blood lymphocytes gated on $CD19^+$ B cells. Filled square, P1. (*C*) BAFF-R expression by B cells from P1, P2, and healthy donors (HD1 and HD2). (*D*) Immunoblot for BAFF-R in whole-cell lysates of EBV lines from P1 and a healthy control. Loading control β-actin. RT-PCR for *TNFRSF13C* mRNA of P1 and a healthy control. (*E*) BAFF-binding to B cells from P1 and P2 compared with those in age- and sex-matched controls HD1 and HD (shaded histogram).

which was located in exon 2 of the *TNFRSF13C* gene of P1 and of P2. As for P1, the phenotypic analysis of blood lymphocytes of P2 showed that the B cells neither expressed BAFF-R nor

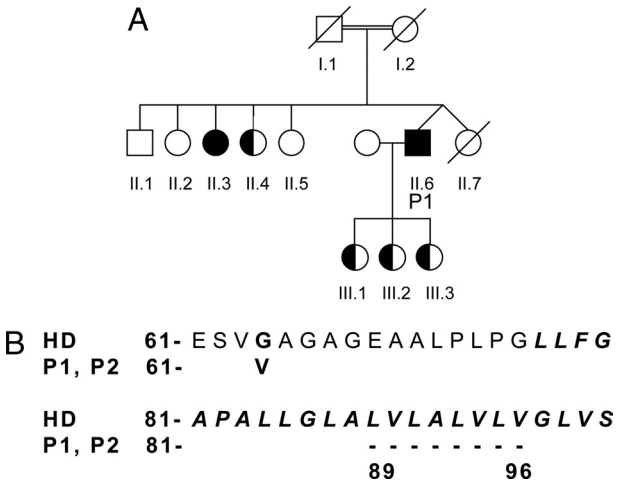

Fig. 2. Genetic analysis of P1 and P2. (*A*) Pedigree of BAFF-R deficient family. Circles, females; squares, males; filled symbols, homozygous individuals; half-filled symbols, heterozygous individuals; crossed-out symbols, deceased individuals. The pedigree shows three generations of the index family. The parental generation is numbered I.1 and I.2, their offspring are II.1–II.7. II.6 represents P1, II.3 represents P2. The children of P1 are labeled III.1–III.3 (*B*) Alignment of reference amino acid sequence, P1 and P2; the TM region is printed in boldface italic type. The deleted AA in the TM region and the G64V exchange of patients P1/P2 are shown below the reference sequence.

were able to bind BAFF (Fig. 1 *C* and *E*). The deletion, which was not found in the genomic DNA from 100 healthy controls, removes a stretch of eight hydrophobic amino acids forming part of the BAFF-R transmembrane region (Fig. 2*B*). In addition to del89–96, P1 and P2 also had a homozygous exchange of two consecutive base pairs resulting in the amino acid exchange of G64V, a previously reported polymorphism (15). Sibling II.4 and all offspring of P1 were heterozygous carriers of del89–96. Subsequently we sequenced the coding regions and the exon-intron boundaries of the three *TNFRSF13C* gene exons in all other 137 CVID patients included in this study and revealed several known *TNFRSF13C* polymorphisms (15) but no other patients carrying obvious disease-causing mutations.

Lymphocyte Phenotyping of Human BAFF-R Deficiency. Phenotypic analysis of blood B cells from P1 and P2 over a period of more than 4 years showed a severe and persistent B lymphopenia in both percentage and absolute numbers, ranging from 1–2% (P1) to 2.8–3.1% (P2) of lymphocytes (normal range, 6–19% in 50 healthy controls) and 10–28 cells/μl (P1) (normal range, 100–500 cells/μl), respectively (Table S1 and Fig. 3*A*). In both patients, the percentage of $CD10^+$ transitional cells was increased (P1, 45%; P2, 21%; Fig. 3 *B* and *C* and Fig. S1) compared with controls (2.5–4.5%), and the absolute numbers were high but still within the normal range. The population of IgM^+ $CD27^+$ marginal zone B cells was much smaller (3%, P1; 7%, P2 vs. 23–26%) and class-switched memory B cells were present but reduced to approximately 7% (P2) and 6% (P1) of B cells (HD 10–20%, Fig. 3*D*). TACI expression by $CD27^+$ B cells was much weaker than in controls (Fig. S2*A*). To exclude TACI deficiency, we tested TACI expression and ligand binding using the EBV B-cell line of P1. Because the affinity of TACI is higher for APRIL than for BAFF, the EBV line showed normal APRIL but weaker BAFF binding. In contrast, a TACI-deficient EBV line derived from a CVID patient carrying a homozygous truncating TACI mutation (16) showed normal BAFF but no APRIL binding, indicating that BAFF-R is the primary receptor for BAFF in humans (Fig. S2*B*).

CD23 expression by transitional and by naïve B cells was comparable to that in controls, whereas CD21 surface levels were slightly lower (Fig. S2*C*).

The distribution of T-cell populations of P1 were similar to controls (Table S1) whereas the number of NK cells was reduced to 26–89/μl (normal range, 90–600/μl). Because it was reported that BAFF can modulate T-cell responses and that BAFF-R might by expressed by activated T cells (17–19), we analyzed BAFF-R expression by resting and activated $CD4^+$ T cells of P1 and of controls. Neither BAFF-R nor TACI were detected on resting or activated $CD4^+$ T cells, suggesting that B cells are the main target for BAFF in humans (Fig. S3).

Medical History, Ig Concentrations, and Vaccination Responses. P1 and P2 were born to a consanguineous marriage (Fig. 2*A*). P1 had a lifelong history of chronic sinusitis and experienced his first case of pneumonia at age 37. At 57 years of age he was diagnosed with CVID after the third case of pneumonia caused by *H. influenzae*. Upper respiratory tract infections were mostly due to *S. pneumoniae* and *H. influenzae*. P2, who is now 80 years old, developed a severe *Herpes zoster* infection at 70 years of age and had two recent episodes of pneumonia but a completely unremarkable earlier medical history. Neither P1 nor P2 developed lymphoproliferative or autoimmune disorders. All other siblings and the offspring of P1 are healthy and have normal B-cell phenotypes except for slightly lower BAFF-R expression in heterozygous carriers.

At the time of diagnosis, P1 had a very low serum IgG concentration of 0.6 g/l (normal range, 7.0–16.0 g/l). The IgA levels remained within the normal range with an average of 2.9

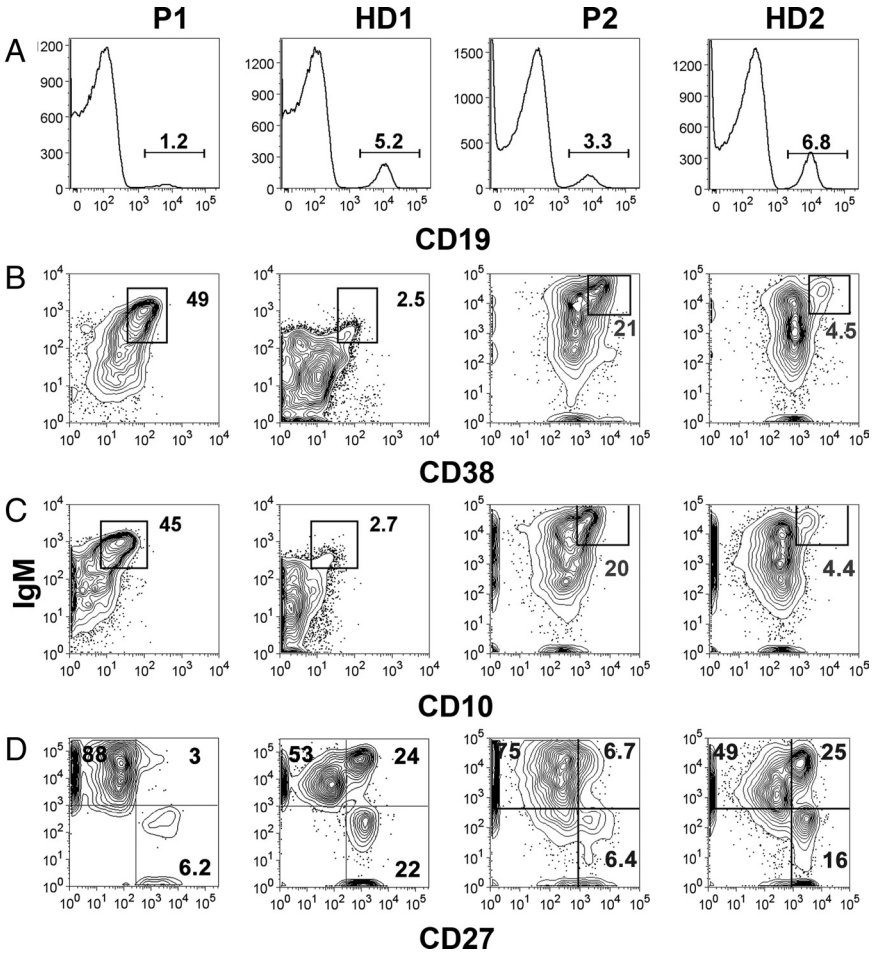

Fig. 3. B-cell phenotype of P1 B cells. (*A*) Percentage of CD19$^+$ B cells for P1, P2, and matched controls, (*B*) CD38, (*C*) CD10, and (*D*) CD27 versus IgM expression by CD19$^+$ gated cells.

g/l over the analyzed period of 7 years. In contrast to IgA, serum IgM concentrations were always less than 0.4 g/l and at many time points even below detection limit (<0.15 g/l). Antibodies against tetanus or diphtheria toxin were not detectable before starting the intravenous Ig substitution. Although P1 experienced several *S. pneumoniae* infections, the serum titers against pneumococcal polysaccharides (PnPS) did not increase at times of infection (data not shown). P2 also had lower IgG levels (5.51 g/l) and low IgM concentrations (0.21 g/l) but normal IgA serum levels (1.1 g/l, Table S2). All other siblings had normal levels of all isotypes (Table S2). It is striking, however, that both BAFF-R–deficient patients had normal levels of IgA as well as IgA$^+$ plasma cells in the gut (P1, Fig. 4*A*), which is a very unusual finding among CVID patients (10, 11), as most CVID patients have neither serum IgA nor intestinal IgA$^+$ plasma cells (20).

Because P2 did not receive ivIg replacement therapy, we were able to monitor T-independent and T-dependent antibody responses after she was vaccinated with pneumococcal polysaccharides and tetanus toxoid (TT). Seven years after her last tetanus vaccination a residual anti-TT IgG titer was detectable (0.12 U/ml), which increased at least 50 times (> 5 U/ml) at 4 weeks after vaccination. In contrast to the T-dependent antigen TT, the T-independent humoral immune response against PnPS was severely impaired, as titers for anti-PnPS IgG binding any of the nine tested serotypes did not increase (Fig. 4*B*). The same result was obtained for P1 after he was immunized with Pneumovax. Although both P1 and P2 had IgM$^+$ B cells carrying somatic mutations that are believed to be precursor cells for antibody responses against encapsulated bacteria (Fig. 4*C*) (21).

Functional Analysis of BAFF-R Deficiency. Ligand binding to BAFF-R activates the alternative nuclear factor–κB (NF-κB) pathway and the processing of NF-κB2 p100 into p52 (22). As expected from the analysis of primary P1 B cells, ST2 cells transduced with a retroviral vector encoding the BAFF-R transmembrane deletion mutant of P1 failed to bind BAFF and to induce NF-κB processing (Fig. S4 *A* and *B*).

As P1 had very low IgG serum concentrations before ivIg substitution, we tested *in vitro* whether the formation of antibody secreting plasmablast-like cells would be impaired. Purified B cells from a matched, adult, healthy donor and from cord blood cells served as controls. The latter were included because P1 had >50% of transitional B cells (Fig. S5) which may react differently to CD40 and IL21R signals than the mature B cells of the adult control. Starting with 50,000 cells per sample, plasmablast formation was tested at different time points by the appearance of CD19^{low} cells expressing high levels of CD38 and CD27 (Fig. S6*A*). Proliferation was determined by counting absolute cell numbers (Fig. S6*B*) and the secretion of immunoglobulins by enzyme-linked immunosorbent assay (ELISA) (Fig. S7). As for the secretion of IgG and IgA, the development of plasmablast-like cells and the proliferative response P1 B cells reacted like cord blood B lymphocytes from the normal donor, likely because both cell preparations contained comparable proportions of transitional B cells (Fig. S5). In comparison to adult and cord blood B cells from normal donors, B lymphocytes of P1 secreted six times more IgM on day 3 and comparable amounts at all later time points (Fig. S7).

Although the genetic defects of P1 and P2 were identical and the immunologic phenotype similar, their different clinical pre-

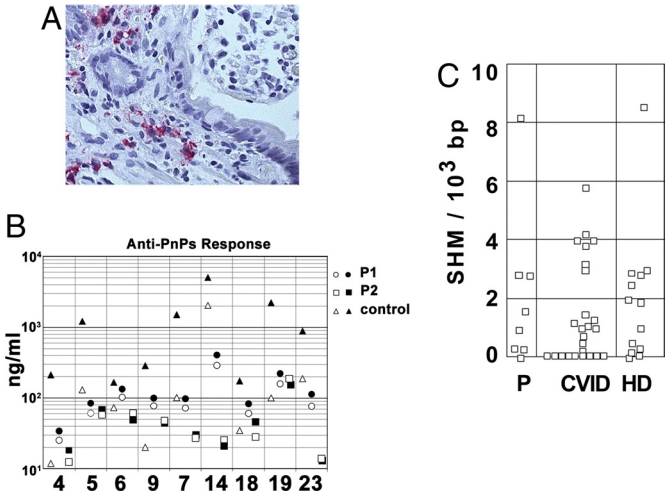

Fig. 4. IgA plasma cells, anti-PnPs response, and somatic hypermutation. (*A*) IgA+ plasma cells stained in red in the lamina propria of the small intestine of P1. (*B*) The antibody titers of P1 and P2 to pneumococcal polysaccharides were analyzed before (open symbols) and after (closed symbols) vaccination with the 23-valent, T-independent pneumococcal polysaccharide vaccine Pneumovax. The IgG titer against the PnP serotypes 4, 5, 6, 7, 9, 14, 18, 19, and 23 was determined by ELISA. Circles, P1; squares, P2; triangles, volunteer vaccinated in parallel serving as healthy control. (*C*) Somatic hypermutations in V3–23E H-chain V regions of μ-heavy chain cDNA amplified from total RNA isolated from P1 and P2, CVID patients ($n = 5$) and HD ($n = 2$). Squares represent the number of mutated bases per 1000 bp/cDNA clone.

sentation was surprising. As B-cell homeostasis as well as serum IgG levels were more severely disturbed in P1, we searched for hypomorphic mutations in X-linked genes that might constitute an additional disadvantage for the development of B cells and humoral immune responses in P1. The DNA sequence of *BTK* did not contain polymorphisms or mutations, and CD40L expression was normal. Searching for a second genetic hit in genes acting in the same pathway as BAFF-R, we also sequenced the *BAFF*, *APRIL*, *TACI,* and *BCMA* genes of P1 but did not find any mutations.

Discussion

Screening our cohort of CVID patients for individuals with potential defects in genes regulating B-cell survival and homeostasis we identified the first two related patients carrying a homozygous deletion within the BAFF-R encoding *TNFRSF13C* gene. The in-frame deletion (del89–96) removed eight hydrophobic amino acids forming part of the BAFF-R transmembrane region. The mutated protein is probably very unstable, as it was neither found in whole-cell lysates of EBV-immortalized cells of P1 nor detected in ST2 cells expressing the BAFF-R deletion mutant from a retroviral expression vector. Proteins with α-helical TM domains are integrated into the lipid bilayer of the ER membrane as nascent polypeptide chains by the translocon (23). Because the deletion (del89–96) removes about half of the TM region, it will most likely change the helical structure of the TM region. Therefore, the mutant BAFF-R either is not recognized by the translocon or it does not interact with the lipid bilayer to allow membrane insertion resulting in protein misfolding and degradation.

In addition to P1 and P2, we found other CVID patients with weaker BAFF-R expression. As we sequenced only the coding regions and the introns but not the regulatory regions of their BAFF-R genes, we cannot exclude the possibility that reduced BAFF-R expression in those patients may result from changes in the promoter or enhancer regions. In addition, epigenetic modification may also influence the accessibility and activity of the BAFF-R gene.

In the case of P1, BAFF-R deficiency correlates with the clinical picture of late-onset common variable immunodeficiency, although P2 did not develop symptoms of antibody deficiency until the age of 70. In both individuals the number of peripheral B cells was reduced more than four times compared with that in age-matched controls, affecting all mature but not the transitional B-cell subsets. The developmental arrests of most B cells at the stage of $CD10^+$ transitional cells strongly indicates that BAFF-R signals support the survival of human transitional B cells while differentiating into mature follicular B cells. In this regard, human BAFF-R deficiency resembles the phenotype found in BAFF-R mutant mice (6–8). The dependence of memory B cells on BAFF-R function seems to be less obvious, as both P1 and P2 had a reduced but still detectable population of class-switched memory B cells (3–7% of $CD19^+$ B cells) correlating also with a strong T-dependent immune response of P2 after immunization with tetanus toxoid. Therefore, in contrast to BAFF-$R^{-/-}$ mice (6, 7), T-dependent immune responses seem to be less reliant on BAFF-R signaling in humans.

Although biopsy samples from P1 revealed a prominent population of IgA^+ plasma cells in the gut, circulating IgA^+ memory B cells, which are formed in lymph nodes, were markedly reduced in P1 and in P2, suggesting that human BAFF-R deficiency impairs the generation of IgA^+ cells in lymph nodes but not in the mucosa-associated lymphoid tissues. Thus, the presence of IgA^+ plasma cells in the gut, which is a very unusual finding among CVID patients (10, 11, 20), emphasizes the BAFF-R–independent differentiation of mucosal IgA^+ B cells in humans, as in mice (6, 24).

Marginal zone B cells eliciting T-independent responses form a first line of defense against encapsulated bacteria (25). In BAFF-R−/− mice marginal zone B cells do not develop (6–8). Although in P1 and P2, IgM^+ $CD27^+$ B cells corresponding to murine MZ B cells were present, their numbers were reduced 6 (P2) to 30 times (P1), correlating well with poor T-independent antibody responses of P1 and P2 to pneumococcal cell wall polysaccharides and infections with *S. pneumonia*. During the initial phase of their reaction against T-independent antigens MZ B cells proliferate strongly in extrafollicular foci, which is even enhanced by CD40 crosslinking (25, 26). This initial part of the extrafollicular response can be mimicked *in vitro* by the polyclonal activation of B cells with CD40L and IL21 (26–28). Applying these activating conditions to P1 B cells, IgM secretion was initially higher and at later time points similar to control cells, indicating that BAFF-R function is not required for CD40/IL21R–induced IgM secretion. *In vitro,* plasmablast development, IgA, and IgG were delayed and not as efficient as for B cells in the adult control, most likely because the majority of P1 B cells were transitional cells, as in cord blood, and only a small proportion had the phenotype of $CD27^+$ marginal zone and switched memory B cells (P1 6%; PBL 35%). Therefore BAFF-R–deficient B cells respond to polyclonal activation like transitional B cells from cord blood and may develop into memory and plasma cells when activated appropriately. However, this does not exclude the possibility that BAFF-R function is needed for the development of plasma cells from IgG+ postmemory B cells residing in the marginal zone of human spleen (29).

In addition, the analysis of $BAFF^{-/-}$ and of BAFF-R−/− mice has clearly demonstrated that B1 B cells can develop independently from BAFF-R function (5–8, 30–37). However, our analysis of blood B cells from both BAFF-R–deficient individuals did not reveal a corresponding B-cell subset in humans. Therefore, the human B-cell compartment either seems not to contain B1 B cells or this subset does not enter the blood stream.

Moreover, phenotypic analysis of B cells from both BAFF-R–deficient individuals, from controls and from cord blood did not reveal TACI expression either by transitional or by naïve follicular B cells, which contrasts with reports from mice describing TACI

expression by both B-cell subsets (37). Because the CD27+ B cells of P1 and P2 expressed less TACI than in controls, TACI expression by $CD27^+$ B cells seems to be regulated by BAFF-R signals, as it has also been found when B cells were stimulated with agonistic anti-BAFF-R antibodies (38). In addition, it was shown that that BAFF-R signals induce CD21 and CD23 expression in murine B cells (39). Our analysis of BAFF-$R^{-/-}$ B cells revealed close to normal CD21 and CD23 expression levels in P2, indicating that $CD21^+$ $CD23^+$ B cells can develop in the absence of BAFF-R in humans. Although P1 and P2 have a very similar B-cell phenotype caused by the same homozygous BAFF-R deletion, both siblings differ significantly in their clinical presentation, which cannot be explained by the lack of individual exposures to pathogens because P2 worked all her life as a nurse. Searching for hypomorphic mutations in genes that might contribute to the clinical phenotype of P1, we could rule out mutations in the genes encoding BTK, BAFF, APRIL, TACI, and BCMA. Other known factors exacerbating CVID, such as chronic infections by EBV or CMV or malignancies, were also excluded. However, gender-related clinical differences between female and male CVID patients have been documented by two previous reports showing that male patients are generally more severely affected (10, 40). Nevertheless, the precise co-modulating factors besides gender accounting for the difference between P1 and P2 remain unknown.

Although the more severe phenotype of P1 exhibits the role of BAFF-R–dependent homeostasis in adults for humoral immune responses against pathogens under normal environmental conditions, the medical history of P2 demonstrates that a BAFF-R–deficient immune system had retained for decades a residual but sufficient potential to develop B cells that can differentiate into antibody-producing plasma cells and provide an efficient host defense against infections also in the absence of BAFF-R function. Therefore it is possible that, within the general population, BAFF-R deficiency is more common than might be estimated from our screening of CVID patients (1/3,000.000–1/6,000.000). The unexpected plasticity of a BAFF-R–deficient humoral immune system in its response against common pathogens was so far not detected in BAFF-R mutant mice. The late onset of disease symptoms itself is also remarkable, and thus BAFF-R deficiency may be regarded as the first immunodeficiency diagnosed primarily in individuals who are in their second half of life. Impaired B-cell–related immunity is an important factor for the increased susceptibility to infections in elderly persons [29]. The search for factors contributing to the immunological defects in this increasing patient population is of great socioeconomic importance, given the current demographic development in the western hemisphere. Thus our findings may also spur research on BAFF/BAFF-R defects in the elderly population in general.

After the monogenetic defects in *ICOS*, *TACI,* and *CD19* (16, 41, 42), *TNFRSF13C* (BAFF-R) becomes the fourth genetic defect attributed to a CVID-like phenotype. It is characterized by a highly variable penetrance ranging from a late-onset severe immunodeficiency to a mild form of hypogammaglobulinemia. Irrespective of these clinical differences, our data demonstrate that BAFF-R plays an important role in the differentiation of transitional into follicular B cells and in the maintenance of normal concentrations of serum IgG in humans. The triad of low peripheral B-cell numbers, an increased ratio of transitional B cells, and intact IgA production *in vitro* and *in vivo* are hallmarks of this defect and might serve as a simple diagnostic algorithm to identify patients with a potential defect in the BAFF-R pathway in the future. Despite the differences between human and murine BAFF-R deficiencies, the murine model shares sufficient similarities to serve in preclinical studies as a tool for agents interfering with Baff–Baff-R interactions, whereas the naturally occurring human monogenetic defects remain highly valuable models in the anticipatory evaluation of new therapies.

Patients and Methods

Patients. All CVID patients were treated at the University Medical Center Freiburg. Informed written consent was obtained from each individual before participation under the internal ethics review board approvals 239/99 and 78/2001.

Flow-Cytometric Analysis. Peripheral blood or cord blood cells were purified by Ficoll gradient centrifugation and stained in a total volume of 25 μl with the following antibodies at optimal concentrations: anti-CD10 PE or FITC, anti-CD21-FITC or -PE, anti-CD23-PE, anti-CD24-PE, anti–CD38-FITC, PE, or PerCP-Cy5.5, anti-CD19-PC7, anti-CD27-FITC or PE, anti-IgD-PE or bio, anti-TACI PE (all BD PharMingen) and anti-IgM-Cy5 (Jackson ImmunoResearch Laboratories). BAFF-R expression was detected by polyclonal rabbit-anti-human BAFF-R (R&D Systems) or PE-conjugated monoclonal anti-BAFF-R antibody 8A7 (eBiosciences) or 11C1 (PharMingen). Binding studies were performed using recombinant human FLAG-tagged BAFF (Alexis Biochemicals), polyclonal rabbit-anti-FLAG IgG (Sigma-Aldrich) and PE-conjugated, cross-absorbed goat anti-rabbit antibodies (Jackson ImmunoResearch Laboratories). For lymphocyte samples, more than 10^4 $CD19^+$ cells were collected on a FACS Canto II (Becton Dickinson) and analyzed using FlowJo software (Tree Star Inc.). Patients were always analyzed in parallel to matched healthy control samples. Cord blood was obtained from Cellgenix (Freiburg, Germany).

Sequence Analysis of BAFF-R. The primers and conditions to amplify and sequence the 3 exons of *TNFRSF13C* have previously been described (16). After separation on an ABI Prism 377 DNA Sequencer (PE, Applied Biosystems), data were analyzed with Sequencher version 3.4.1 (Gene Codes Corp.).

Detection of SHM in the IgH V Regions. Total RNA was isolated from PBL after TRIzol (Invitrogen) extraction and purification over RNeasy columns (Qiagen). cDNA was amplified with Pfu polymerase (Stratagene/Agilent) using VH3–23 (43) and Cμ-specific primers (44). SHM were identified in VH3–23 containing fragments cloned into into PCR-Topo II (Invitrogen).

Histology. After heat-mediated antigen retrieval from deparaffinized and rehydrated duodenal biopsy samples, IgA heavy chains were visualized with rabbit anti-human IgA and streptavidin-biotin complexes using the AUTOSTAINER Plus (DAKO).

Immunoblotting. Proteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were transferred to PVDF membranes (Millipore), incubated with polyclonal rabbit antibodies against BAFF-R (ProSci Inc.), or actin (Santa Cruz), and developed using secondary HRPO-conjugated antibodies from Southern Biotech and ECL substrate (Pierce).

For additional information, please see *SI Text*.

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