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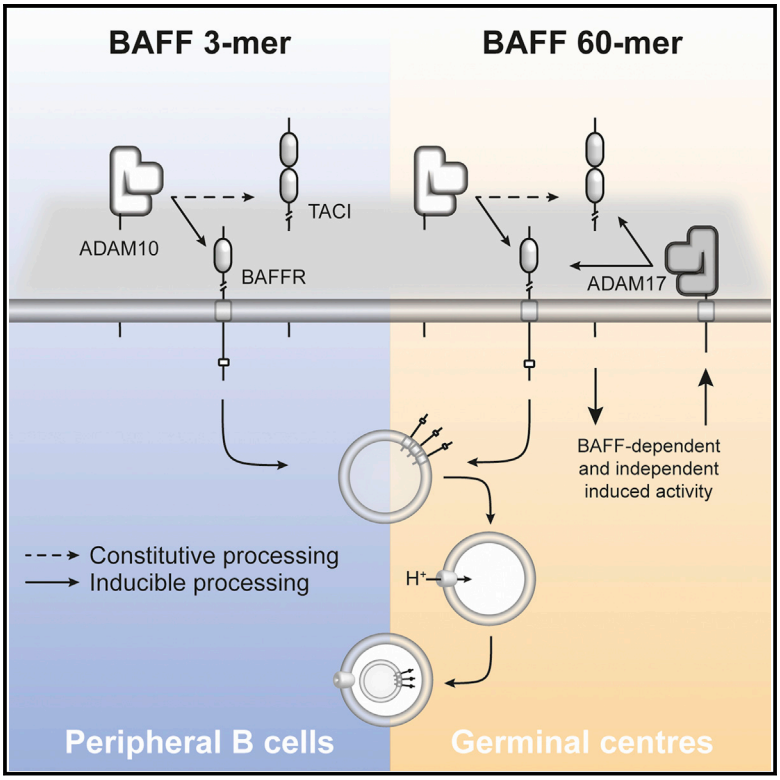
Graphical Abstract



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In Brief

Smulski et al. report that the B cell survival receptor BAFFR undergoes ligand-induced shedding but only in cells co-expressing a second receptor for BAFF called TACI. BAFFR shedding can be performed by ADAM10 in circulating B cells or by ADAM17 in germinal center B cells and limits BAFF-mediated survival signals.

Highlights

- BAFF induces shedding of BAFFR in TACI-positive B cells
- BAFFR shedding limits BAFF-induced signals
- Shedding can be performed by ADAM10 or ADAM17 depending on the context

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BAFF- and TACI-Dependent Processing of BAFFR by ADAM Proteases Regulates the Survival of B Cells

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SUMMARY

B cell activating factor (BAFF) provides B cells with essential survival signals. It binds to three receptors: BAFFR, TACI, and BCMA that are differentially expressed by B cell subsets. BAFFR is early expressed in circulating B cells and provides key signals for further maturation. Here, we report that highly regulated BAFFR processing events modulate BAFF responses. BAFFR processing is triggered by BAFF binding in B cells co-expressing TACI and it is executed by the metalloproteases ADAM10 and ADAM17. The degree of BAFF oligomerization, the expression of ADAM proteins in different B cell subsets, and the activation status of the cell determine the proteases involved in BAFFR processing. Inhibition of ADAM10 augments BAFF-dependent survival of primary human B cells, whereas inhibition of ADAM17 increases BAFFR expression levels on germinal center B cells. Therefore, BAFF-induced processing of BAFFR regulates BAFF-mediated B cell responses in a TACI-dependent manner.

INTRODUCTION

B-lymphocytes are essential components of adaptive immune responses. Developing from precursor cells in the bone marrow, immature B cells enter the spleen where they fully develop into follicular and marginal zone (MZ) B cells. Follicular B cells patrol via circulation and lymph through the whole body searching for pathogens and antigens. Activated by antigen binding to surface IgM and IgD, they proliferate and differentiate in germinal centers into long-lived antibody-secreting plasma cells and memory B cells expressing high affinity IgA, IgE, or IgG antibodies. Most of the MZ B cells are specific for encapsulated bacteria and develop in response to antigen binding into short-lived plasma cells. These B cell subsets express three tumor necrosis factor (TNF)-receptor family members termed BAFFR, BCMA, and TACI. The three receptors regulate the survival of B cells and plasma cells by interacting with BAFF (B cell activating factor of the TNF family). In contrast to BAFFR, BCMA and TACI also bind the closely related ligand called APRIL (a proliferation-inducing ligand) (Kalled, 2002; Dillon et al., 2006; Bossen and Schneider, 2006; Schuepbach-Mallepell et al., 2015). The three receptors are expressed in a subset-specific manner starting with BAFFR in transitional B cells followed by TACI in marginal zone and switched memory B cells and finally by BCMA in plasma cells (Pieper et al., 2013). The differential expression of these receptors during B cell development allows at least three distinct combinations: naive B cells express only BAFFR, marginal zone and switched memory B cells express BAFFR and TACI, and plasma cells express TACI and BCMA. How the combinatorial expression of these receptors may affect the outcome of the signals triggered by BAFF as a common ligand is a puzzling question that we start to address in this work.

Deletion of the BAFF-encoding *Tnfsf13b* gene in mice interrupts B cell development at the stage of transitional B cells causing humoral immunodeficiency (Gross et al., 2001; Schiemann et al., 2001). A similar but slightly less severe phenotype is observed in BAFFR-deficient mice (Shulga-Morskaya et al., 2004). In contrast, overexpression of BAFF promotes the polyclonal expansion of B cells and the development of hypergammaglobulinemia and autoimmunity (Mackay and Schneider, 2009). Also in humans, *BAFFR* deficiency blocks B cell development at the transitional stage. It results in reduced IgG and IgM serum concentrations and impedes T-independent immune responses against pneumococcal cell wall polysaccharides (Warnatz et al., 2009).

TACI is upregulated in activated B cells in response to B cell receptor or Toll-like receptor (TLR) ligands (Groom et al., 2007; Ng et al., 2005) and appears to have opposing roles. On the one hand, TACI negatively regulates B cell homeostasis, because Taci$^{-/-}$ mice have elevated numbers of B cells. On the other hand, TACI seems to promote the differentiation or survival of plasmablasts (Mantchev et al., 2007), as T-independent humoral responses are severely reduced in Taci$^{-/-}$ mice

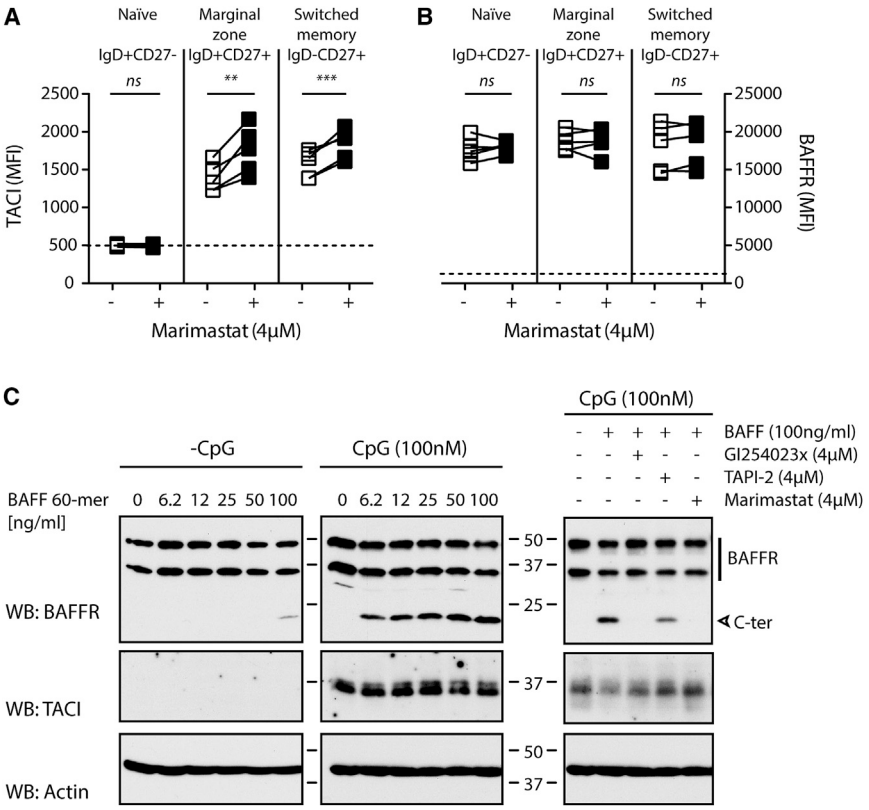

Figure 1. BAFF Induces BAFFR Processing in Primary Human B Cells

(A) Human B cells were incubated over night with the pan-metalloprotease inhibitor marimastat. TACI surface levels were determined by flow cytometry. CD19$^+$ B cell subsets were identified according to the expression of IgD and CD27 as follows: naive, IgD$^+$ CD27$^-$; marginal zone, IgD$^+$ CD27$^+$; switched memory B cells, IgD$^-$ CD27$^+$.

(B) Cells were treated as in (A) and analyzed for BAFFR surface expression. Samples were from five independent healthy donors. Significant differences were analyzed by a paired t test. The gating strategy and survival rate are outlined in Figures S1A–S1C.

(C) Western blot analysis of whole cell lysates of resting (left) or CpG-activated (100 nM, middle) CD27-negative human B cells treated overnight with increasing concentrations of BAFF 60-mer revealed the accumulation of a 22 kDa BAFFR C-terminal fragment (arrowhead, C-ter). The ADAM10 inhibitor GI254023x (4 μM) and the pan-metalloprotease inhibitor marimastat (4 μM) but not by the ADAM17 inhibitor TAPI-2 (4 μM), block processing completely (right panel). One representative blot out of three independent experiments is shown. For BAFFR, two signals migrating at ~50 and 36 kDa were detected.

(Yan et al., 2001; von Bülow et al., 2001). Moreover, BAFF and APRIL signals relayed by TACI augment the survival of primary B cells ex vivo (Treml et al., 2007; Katsenelson et al., 2007; Bossen et al., 2008), whereas simultaneous engagement of Toll-like receptors and TACI sensitizes marginal zone B cells to Fas-induced apoptosis (Figgett et al., 2013). Finally, depletion of BAFF or BAFF and APRIL in humans treated with monoclonal anti-BAFF antibodies or with TACI-Ig decoy receptors strongly reduces the number of circulating B cells (Stohl et al., 2012; Tak et al., 2008; Wallace et al., 2009).

Recently, it was reported that TACI is constitutively shed by a disintegrin and metalloprotease (ADAM) 10, releasing a soluble fragment with decoy receptor activity (Hoffmann et al., 2015). BCMA is constitutively shed from the cell surface by γ-secretase activity without the need for an initial processing by another protease (Laurent et al., 2015). Correlating with the disease activity, increased concentration of soluble forms of TACI and BCMA were found in the spinal fluid of multiple sclerosis patients as well as in the serum from systemic lupus erythematosus patients. In both cases, shedding reduced the receptor-dependent activation of nuclear factor κB (NF-κB), indicating a negative impact on signaling (Hoffmann et al., 2015; Laurent et al., 2015). However, it remains elusive whether shedding can be triggered or enhanced by any specific signal.

In our study, we report that BAFFR is processed in a regulated manner and not constitutively like TACI and BCMA. BAFFR is proteolytically cleaved after BAFF binding, but only in cells co-expressing TACI. Moreover, different forms of the ligand BAFF triggered different processing pathways leading to the proteolytic cleavage of BAFFR and TACI. BAFF 3-mers induced processing of BAFFR by ADAM10 and did not affect the processing of TACI, whereas BAFF 60-mers activated BAFFR and TACI cleavage by ADAM10 and by ADAM17. In resting and in TLR9-activated human B cells, ADAM10 is the main protease that cleaves BAFFR in response to BAFF binding, whereas ADAM17 seems to be responsible for the processing of BAFFR in B cells from the dark zone of germinal centers. Inhibition of ADAM10 activity augmented BAFF-dependent survival and IgM secretion, whereas inhibition of ADAM17 restored BAFFR expression on the cell surface of B cells from the dark zone of germinal centers. Therefore, BAFF-dependent processing of BAFFR is a mechanism that regulates BAFFR surface levels on B cells and thus BAFFR mediated B cell survival.

RESULTS

BAFF Induces BAFFR Processing in Primary Human B Cells

Because TACI and BCMA are shed constitutively (Hoffmann et al., 2015; Laurent et al., 2015), we tested whether BAFFR is also processed constitutively by metalloproteases. B cells were isolated from the blood of healthy donors, incubated overnight with the pan-metalloprotease inhibitor marimastat, and analyzed by flow cytometry. Whereas TACI expression increased on the surface of IgD$^+$ CD27$^+$ (marginal zone/IgM memory, MZ) and IgD$^-$ CD27$^+$ (switched memory) B cells (Figure 1A) BAFFR expression remained unchanged in all B cell subsets, indicating that BAFFR was not constitutively processed (Figure 1B). As

BAFF binding to BAFFR might change its conformation and expose potential cleavage sites to proteases, we tested if BAFFR would be cleaved in the course of BAFF treatment. Resting B cells were incubated overnight with increasing concentrations of BAFF 60-mers and analyzed by western blot (Figure 1C). Only at BAFF concentrations $\geq$100 ng/mL BAFFR processing was detectable as a new signal derived from the C-terminal region of BAFFR migrating at 22 kDa (Figure 1C, left). However, activation of TLR9 with CpG strongly enhanced BAFF-dependent BAFFR processing as the C-terminal fragment was detected already at a BAFF 60-mer concentration as low as 6.25 ng/mL (Figure 1C, middle). Notably, the increased sensitivity of BAFFR to BAFF-dependent proteolytic cleavage correlated with the upregulation of TACI (Figure 1C). Because BAFF-induced processing of BAFFR was completely blocked by the pan-metalloprotease inhibitor marimastat and by the ADAM10 inhibitor GI254023x, but not by the ADAM17 inhibitor TAPI-2 (Figure 1C, right), ADAM10 seemed to be responsible for BAFF-induced BAFFR processing in CpG-activated human B cells.

BAFF-Induced BAFFR Processing Depends on TACI

Because CpG stimulation led to strong upregulation of TACI (Figure 1C) (Treml et al., 2007), we tested whether processing of BAFFR required the co-expression of TACI. Two different cellular models were used: the Burkitt's lymphoma cell line BJAB and the EBV transformed B cell line IM9. BJAB cells express only BAFFR, but not TACI, whereas IM9 cells express both receptors. BAFF treatment of BJAB cells did not change total BAFFR protein levels whereas they clearly decreased over time in BAFF-treated BJAB cells transduced with TACI (BJAB-TACI) (Figure 2A). In a similar way, total BAFFR and TACI levels decreased in BAFF-treated IM9 cells in a time-dependent manner, whereas BAFFR was not processed in TACI-knockout IM9 cells (IM9-TACI KO) (Figure 2B). After 24 hr, most of BAFFR had been processed, and only after 72 hr, the initial BAFFR protein levels were reached again. A similar kinetic was observed in both cell lines (Figures 2C and 2D).

Soluble BAFF assembles into trimers and into 60-mers and both forms can bind to BAFFR and TACI (Cachero et al., 2006; Bossen et al., 2008). We therefore analyzed if the degree of BAFF oligomerization had an effect on BAFFR processing. In addition, we tested if BAFFR processing required the engagement of both receptors by using APRIL, which only binds to TACI but not to BAFFR. Treatment of BJAB-TACI cells with APRIL did not change BAFFR levels, showing that ligand binding to TACI alone did not trigger BAFFR processing (Figures 2E and 2F, left). Although both forms of BAFF, the trimer and the 60-mer, induced BAFFR processing (Figures 2E and 2F, right), only the 60-mer enhanced processing of TACI above the levels of constitutive shedding in IM9 cells (Figures 2B and 2F). TACI levels remained unchanged in BJAB-TACI cells, possibly because the high expression levels of transduced TACI masked processing occurring at the cell surface (Figures 2A and 2E). qPCR showed that BAFF treatment neither changed the mRNA levels of BAFFR nor of TACI (Figure 2G). Although the time- and dose-dependent decrease of total BAFFR protein levels was readily detected, the C-terminal 22 kDa BAFFR fragment observed in primary B cells was not found in these cell lines. To study the fate of the C-terminal fragment released during processing, we tested several drugs affecting endocytosis and vesicular transport. Blocking of lysosomal activity using the vacuolar-type H^+-ATPase-specific inhibitor bafilomycin A1 led to the dose-dependent accumulation of a BAFFR C-terminal fragment in BAFF-treated BJAB-TACI cells (Figure S2A), corresponding to the signal detected in primary B cells (Figure 1C). This 22 kDa C-terminal region of BAFFR includes most likely the transmembrane segment, because it was enriched in the membrane and not in the soluble subcellular fraction of BJAB-TACI cells (Figure S2B). Moreover, it was also detected when membrane fractions of BAFFR-transduced EBV6 cells were treated in vitro with rhADAM10 (Figure S2C). Binding of BAFF trimers to BAFFR-YFP fusion proteins expressed in BAFFR-deficient EBV6 cells decreased BAFFR surface expression and reduced the fluorescence of EYFP. When bafilomycin A1 was included, only BAFFR surface expression, but not the YPF signal, decreased, supporting the hypothesis that the inhibition of lysosomal vesicle transport prevented the degradation of the C-terminal fragment (Figure S2D).

To demonstrate that BAFFR is processed on the cell surface, we compared cell surface BAFFR expression with total BAFFR protein levels following BAFF treatment. However, binding of anti-BAFFR antibodies to the extracellular portion of BAFFR was inhibited by receptor-bound BAFF. Therefore, ligand was removed by a brief acid treatment ("acid elution") originally developed for MHC-bound peptides (Purcell, 2004; Fortier et al., 2008). Washing cells for 1 min at low pH efficiently eluted BAFFR-bound BAFF trimers but not BAFF 60-mers (Figures 2H and S3A), whereas BAFF bound to TACI was not removed (Figure S3B). Thus, we used this technique to analyze BAFFR processing induced by BAFF trimer but not by BAFF 60-mer.

As expected, BAFFR was not processed in TACI-negative BJAB cells (Figure 2I), but when BJAB-TACI cells were incubated with BAFF trimers, we observed a similar decrease of cell surface BAFFR and of total BAFFR levels (Figures 2I, S3C, and S3D), suggesting that BAFFR was processed on the cell surface upon BAFF binding. The incubation with BAFF 60-mers interfered with the visualization of cell surface BAFFR (Figure S3E) allowing the detection of BAFFR processing only by western blot (Figure S3F). Similar to BJAB-TACI cells, binding of BAFF trimers reduced BAFFR surface expression on TACI-expressing IM9 and EBV1 cells but not on TACI-KO IM9 cells, or on a EBV line homozygous for the TACI-S144X mutation (Figures S3G and S3H).

Taken together, these results show that BAFF binding to BAFFR induced the processing of the receptor on the surface of TACI-expressing B cell lines generating a C-terminal fragment that is degraded by the lysosomes.

Primary Immunodeficiency-Associated Mutations in BAFFR and TACI Affect BAFF-Induced Processing

Because BAFFR was processed in a BAFF-dependent manner in the presence of TACI, we analyzed if *BAFFR* and *TACI* mutations found in the human population might affect BAFF-induced processing of these two receptors. To this end, we used EBV-immortalized B cell lines derived from primary immunodeficiency patients carrying previously characterized mutations in BAFFR or TACI. EBV3 carries a homozygous nonsense mutation in

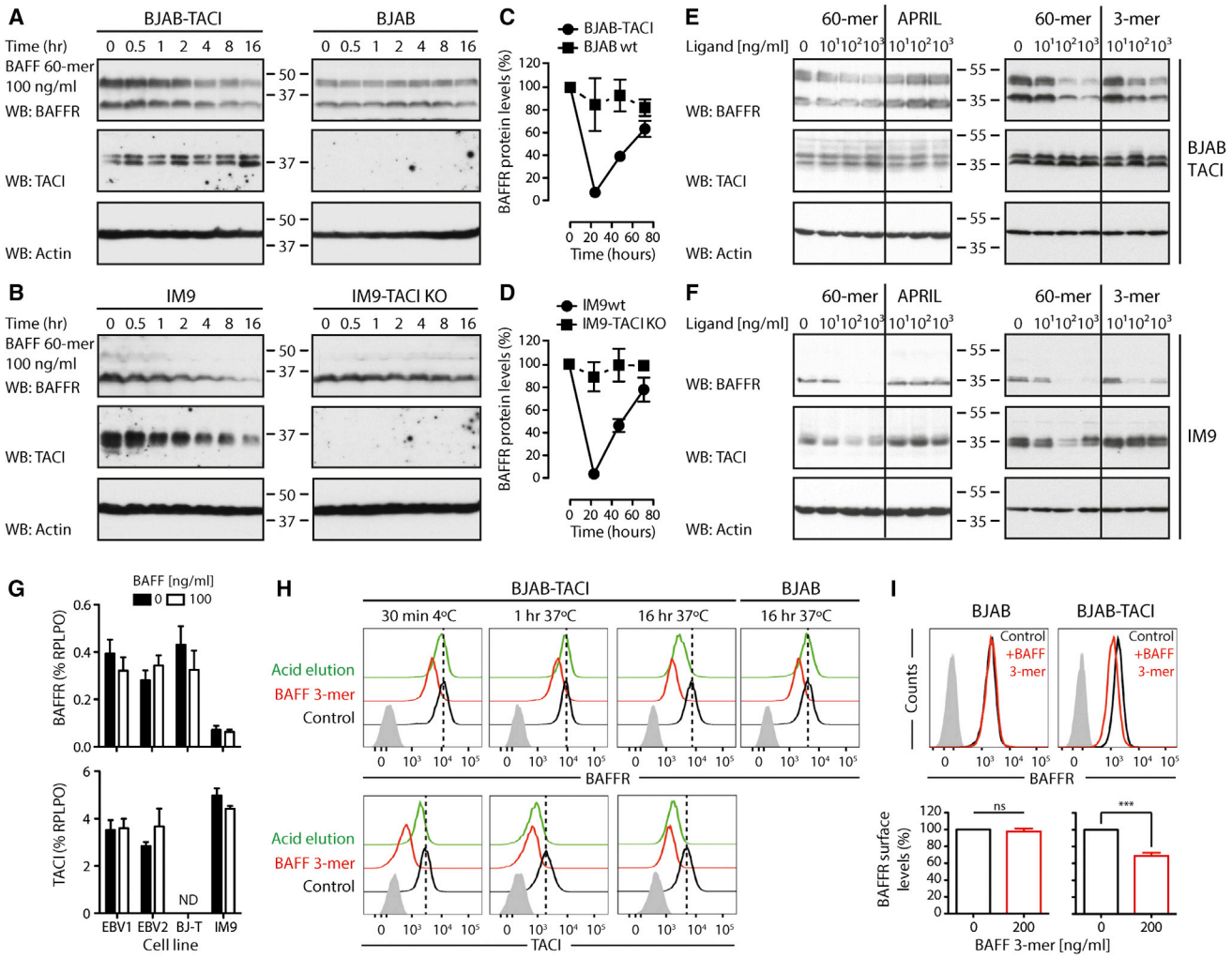

Figure 2. BAFF-Induced BAFFR Processing Depends on TACI

(A) BJAB-TACI or BJAB WT cells were treated with 100 ng/mL BAFF 60-mer and analyzed at the indicated time points by western blot of whole cell lysates.

(B) Same as (A) for IM9 and IM9-TACI KO cells.

(C) BAFFR protein levels were evaluated by western blot at 0, 24, 48, and 72 hr after addition of 100 ng/mL of BAFF 60-mer in BJAB and in BJAB-TACI cells (mean values ± SEM of densitometric analysis of two independent experiments).

(D) Same as (C), but for IM9 and IM9TACI-KO cells.

(E) Overnight treatment with BAFF 60-mer or APRIL (left panel), BAFF 60-mer, or BAFF 3-mer (right panel) at the indicated doses.

(F) Same as (E) but for IM9 cells. The images in (A), (B), (E), and (F) display one out of five representative experiments.

(G) qPCR of BAFFR (top) and TACI (bottom) mRNA in B cell lines stimulated or not with BAFF 60-mer at 100 ng/mL. EBV1 (healthy donor, HD), EBV2 (HD), BJAB-TACI (BJ-T), and IM9. Expression levels are normalized to the housekeeping gene *RPLP0*. ND, not done.

(H) Incubation of BJAB-TACI cells with 100 ng/mL of BAFF 3-mer inhibits staining with anti-BAFFR mAb as shown by weaker FACS signals (red histogram plot). Signals are restored after acid elution (pH 2.4, 1 min: green histogram plot).

(I) FACS plots of BJAB and of BJAB-TACI incubated with 200 ng/mL of BAFF trimer for 16 hr. Acidic elution was performed prior to antibody staining. Mean values and SEM of BAFFR surface levels of three independent experiments are shown. Significant differences were calculated by a paired t test.

TACI (S144X, encoded by rs104894650) preventing TACI expression (Salzer et al., 2005). Like the TACI-negative BJAB or IM9 TACI-KO lines, TACI S144X EBV3 cells did not process BAFFR after BAFF binding (Figure 3A, also shown in Figures S3G and S3H). To define if BAFFR processing requires ligand binding to BAFFR and TACI, we used EBV lines that express mutant forms of BAFFR or TACI interfering with BAFF binding. EBV4 carries a homozygous missense mutation in TACI (C104R, rs34557412) preventing the formation of a cysteine bridge in its ligand-binding domain (Bacchelli et al., 2011). When treated with BAFF, this cell line neither processed BAFFR nor TACI. EBV5 contains a homozygous missense mutation in BAFFR (P21R) encoded by SNP rs77874543. It affects the pre-ligand assembly domain, disturbs BAFFR multimerization, and reduces BAFF binding (Pieper et al., 2014). When treated with BAFF, this cell line did not process BAFFR but showed enhanced

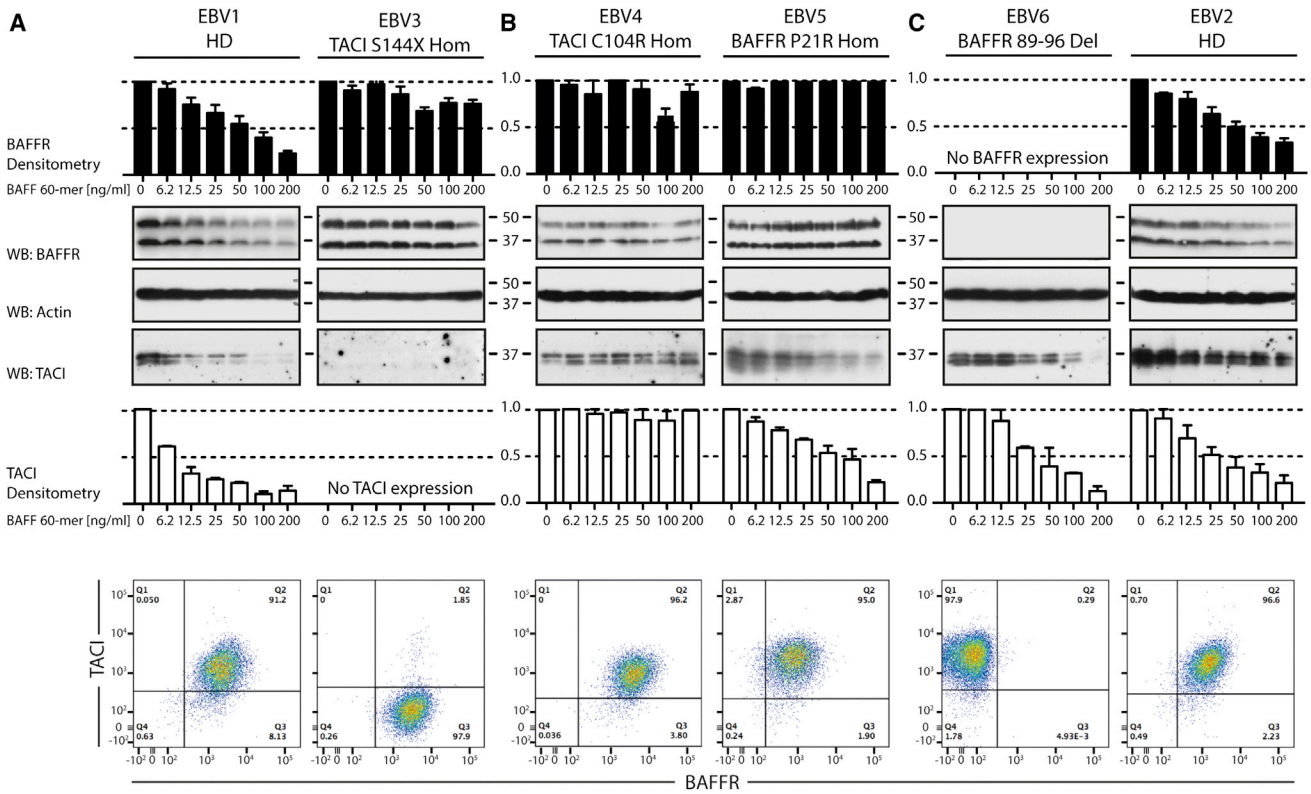

Figure 3. Primary Immunodeficiency-Associated Mutations in BAFFR and TACI Affect BAFF-Induced Processing

(A) Overnight treatment with increasing doses BAFF 60-mer and analysis of BAFFR and TACI protein levels by western blot of whole cells lysates of EBV1 (HD, healthy donor) and EBV3 (TACI-negative S144X homozygous) cells.

(B) Same as (A) but for EBV4 (TACI C104R homozygous; no ligand binding) and EBV5 (BAFFR P21R homozygous; deficient for BAFFR multimerization, reduced ligand binding).

(C) Same as (A) but for EBV6 (homozygous deletion BAFFR removing amino acids 89–96) and EBV2 (HD). Average values of densitometric analysis of BAFFR (top) and TACI (bottom) protein from at least two independent experiments (± SD). FACS plots display TACI and BAFFR surface expression levels for each cell line.

processing of TACI. Because both mutations prevented BAFFR processing (Figure 3B), BAFFR cleavage requires BAFF binding to functional BAFFR and TACI molecules. Moreover, ligand-induced processing of TACI occurred in the P21R BAFFR line EBV5 and in the BAFFR-deficient EBV line EBV6 (Figure 3C), indicating that BAFF can enhance TACI processing independent of BAFFR expression or function.

BAFFR Is Processed by ADAM10 and by ADAM17

ADAM10 and ADAM17 can process a variety of different proteins. Many of these are common targets for both proteases. Therefore, to define the role of ADAM10 and ADAM17 more precisely, we used specific ADAM inhibitors and knockout cell lines. Inhibition of ADAM10, but not of ADAM17, activity prevented BAFF trimer-induced BAFFR processing in BJAB-TACI cells (Figure 4A). Likewise, BAFFR was not processed in BJAB-TACI cells in which the *ADAM10* alleles had been knocked out by CRISPR/Cas9-mediated mutagenesis (Figure 4B), whereas knockout of *ADAM17* still allowed BAFFR processing.

Thus, binding of BAFF trimers induced ADAM10- but not ADAM17-dependent processing of BAFFR. This pattern changed when BAFF 60-mers instead of trimers were used, because they induced processing by ADAM10 and by ADAM17, which was only prevented by the pan-metalloprotease inhibitor marimastat (Figure 4C) and by knocking out both *ADAM10* and *ADAM17* (Figure 4D). Neither the inhibition of both proteases (Figure 4E) nor their genetic inactivation (Figures 4F and 4G) changed the surface expression levels of BAFFR, strengthening our observation made with primary B cells (Figure 1B) that BAFFR is not processed constitutively. In contrast, TACI surface levels strongly increased in the presence of ADAM10 inhibitors (Figure 4E) as well as on ADAM10 knockout cells (Figure 4F). This result confirms that TACI is constitutively shed by ADAM10 (Hoffmann et al., 2015). Because BAFF 60-mer can activate TACI signaling while trimeric BAFF cannot (Bossen et al., 2008), we reasoned that BAFF 60-mers might activate ADAM17 in a TACI-dependent manner. Incubation of BJAB-TACI cells with BAFF 60-mers strongly enhanced ADAM17 surface expression within 3 hr (Figure 4H), whereas BAFF trimers or the treatment of TACI-negative BJAB cells with BAFF 60-mers had no effect. Under the same conditions, ADAM10 surface levels remained unchanged (Figure 4H). This suggests that cell surface levels of

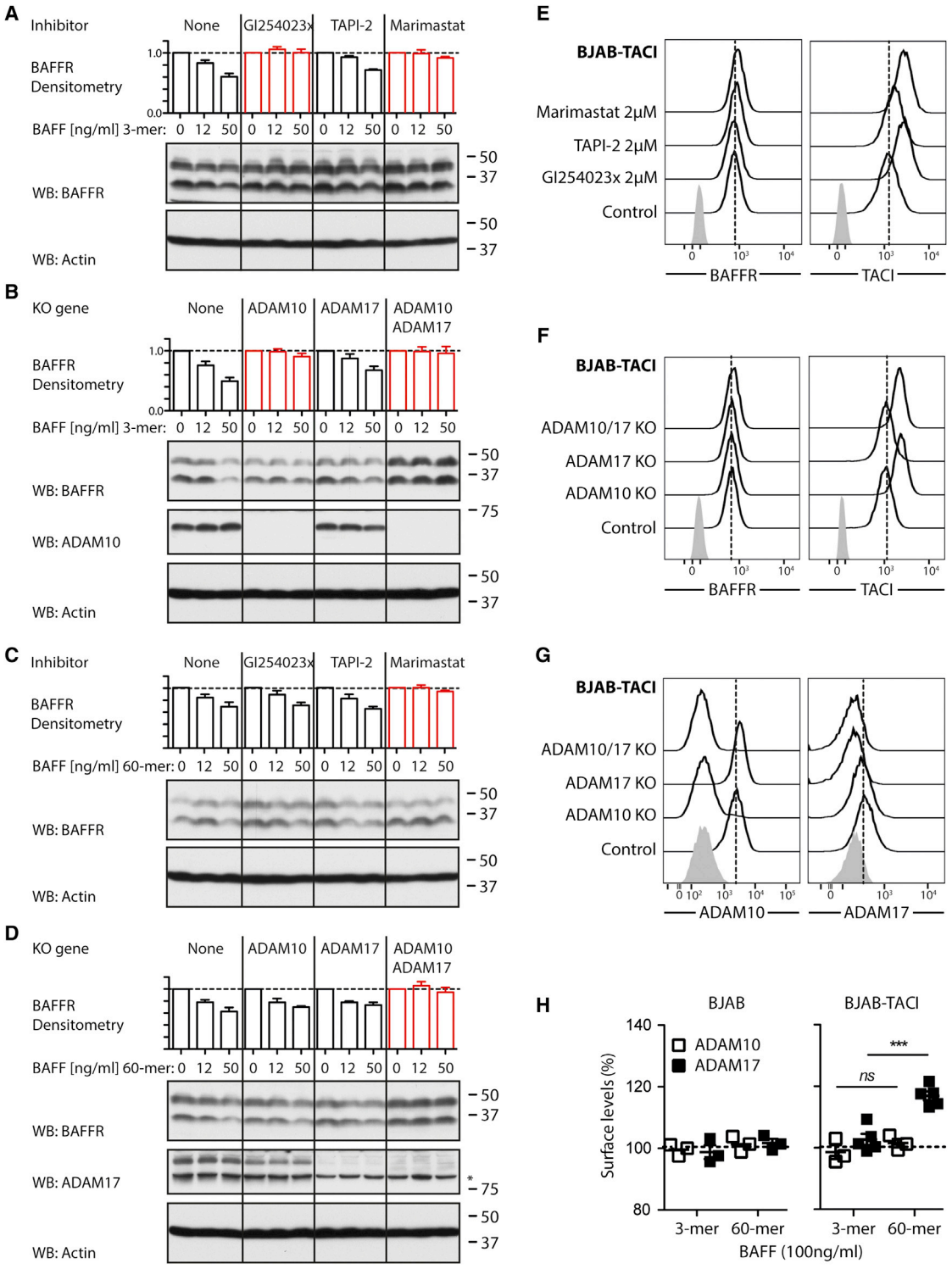

Figure 4. BAFFR Is Processed by ADAM10 and by ADAM17

(A) BJAB-TACI cells were incubated over night with increasing amounts of trimeric BAFF and with or without the ADAM10 inhibitor GI254023x, the ADAM17 inhibitor TAPI-2, or the pan-metalloprotease inhibitor marimastat. Processing is shown by decreased BAFFR signals calculated from densitometric analysis of western blot images.

(B) BJAB-TACI, BJAB-TACI ADAM10 KO, ADAM17 KO, and ADAM10/17 double KO cells were incubated overnight with BAFF trimers and analyzed as outlined in (A).

(C) Experiments were performed as in (A) but with BAFF 60-mers instead of trimers.

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ADAM17 can be regulated by TACI in a BAFF-dependent manner.

Therefore, ADAM10-dependent processing of BAFFR requires binding of BAFF trimers or 60-mers to BAFFR and TACI. It differs from ADAM10-mediated shedding of TACI, which is processed constitutively independent of BAFF binding and BAFFR expression. In addition, binding of BAFF 60-mers induces proteolytic cleavage of BAFFR by ADAM17 in a ligand-dependent way.

BAFFR Processing Can Be Triggered in a Ligand-Independent Manner

The proteolytic activity of ADAM17 is rapidly induced by cellular activators (Le Gall et al., 2010) including phorbol-12-myristate-13-acetate (PMA), a strong activator of protein kinase C (PKC). We therefore tested if the PKC-dependent activation of ADAM17 by PMA would suffice for BAFFR processing in the absence of BAFF. Activation of ADAM17 with PMA for 1 hr led to the processing of BAFFR as well as of TACI in ADAM17-expressing BJAB-TACI but not in *ADAM17* KO cells (Figure S4A). The proteolytic activity of ADAM10 can be enhanced by calcium flux (Hundhausen et al., 2007). Therefore, we tested if activation of ADAM10 by ionomycin would suffice for BAFFR processing in the absence of BAFF. While BAFFR levels remained constant, TACI expression decreased in ADAM10 competent cells (Figure S4B) showing that although the activation of ADAM10 enhanced shedding of TACI, it did not induce processing of BAFFR. Accordingly, ionomycin treatment of primary B cells had no effect on BAFFR surface expression but significantly reduced TACI levels in $IgD^{+}CD27^{+}$ (marginal zone) and $IgD^{-}CD27^{+}$ (switched memory) B cells (Figure 5A).

Ligand-independent, PMA-induced processing of BAFFR was detected in all primary B cell subsets by the reduction of BAFFR surface levels (Figure 5B). Addition of the ADAM17 inhibitor TAPI-2 to PMA-activated B cells blocked PMA-induced BAFFR processing. A similar effect was observed for TACI surface levels. Moreover, decreased levels of BAFFR and TACI on the cell surface correlated with increased levels of soluble BAFFR and TACI in supernatants (Figures 5C and 5D). Production of soluble BAFF in each condition showed very low levels following PMA treatment, slight increase by ionomycin and close to 4 ng/mL BAFF in PMA plus ionomycin treatment (Figure 5E). This increase inversely correlated with the detection of soluble TACI, but it did not change the detection of soluble BAFFR.

These results show that BAFFR can be processed by ADAM10 and/or by ADAM17, depending on the mode of activation, releasing a soluble receptor. Only PKC-dependent activation of ADAM17 allowed BAFFR cleavage in a ligand-independent way.

To identify the minimal requirements for BAFFR processing, we compared the cleavage of membrane-bound, full-length BAFFR and of recombinant human BAFFR-IgG1 Fc fusion protein (rhBAFFR-Fc) by active recombinant human ADAM10 and ADAM17 (rhADAM10/17). RhADAM10 cleaved membrane-bound BAFFR only when co-incubated with BAFF (Figure S5A) but it did not cleave rhBAFFR-Fc. Membrane-bound BAFFR was also cleaved by rhADAM17 in the presence of BAFF, while rhBAFFR-Fc was not processed (Figure S5B). This suggests that BAFFR is cleaved in the extracellular domain in the immediate vicinity of the transmembrane domain, a region that is not present in the BAFFR-Fc construct. Alternatively, the insertion into the plasma membrane might be required for the recognition of BAFFR as an ADAM substrate.

Taken together, these results suggest that BAFF binding allows ADAM-mediated cleavage of BAFFR within its extracellular domain close to the transmembrane segment.

BAFFR Processing Regulates BAFF-Induced B Cell Survival

So far, our experiments showed that BAFF binding led to BAFFR processing in TACI-expressing B cells. To address the question if BAFFR processing affects the response of B cells to BAFF, we analyzed the survival of human B cells in the presence of ADAM10 or ADAM17 inhibitors. Purified B cells were cultivated for 3 days with increasing concentrations of BAFF 60-mer alone or in combination with the ADAM10 inhibitor GI254023x or with the ADAM17 inhibitor TAPI-2. Addition of GI254023x significantly increased BAFF-supported B cell survival when compared to BAFF alone, whereas TAPI-2 had no effect (Figure 6A). Likewise, BAFF-dependent survival and IgM secretion increased when B cells were activated in the presence of GI254023x with CpG or with anti-IgM and CpG (Figures 6B and 6C). Therefore, processing might limit BAFFR signaling in resting and activated B cells.

In addition to BCR and TLR signals, B cells are activated in germinal centers (GC) by T-helper cells, inducing proliferation in GC dark zones (DZ) and selection and differentiation into memory B cells and plasma cells in light zones (LZ) (McHeyzer-Williams et al., 2011). Within GCs, BAFF is produced by follicular dendritic cells (Suzuki et al., 2010) as well as by T-follicular helper cells (Treml et al., 2007) thus regulating the output of autoreactive B cells in mice and humans (Mackay and Schneider, 2009;

(D) BAFFR processing was analyzed as in (B) but with BAFF 60-mers instead of trimers. Bar graphs represent means and SEM of signal intensities of three independent experiments normalized using actin expression as internal standard. Red bar graphs indicate inhibition of BAFFR processing. The loss of ADAM10 and ADAM17 expression in the corresponding cell lines was verified by western blot.

(E) BJAB-TACI cells were incubated overnight in the presence of metalloprotease inhibitors GI254023x, TAPI-2, or marimastat. BAFFR and TACI expression were analyzed by flow cytometry and are represented by histogram profiles.

(F) BJAB-TACI, BJAB-TACI ADAM10 KO, ADAM17 KO, and ADAM10/17 double KO cells were analyzed by flow cytometry for BAFFR and TACI surface expression.

(G) Flow cytometry analysis of ADAM10 and ADAM17 surface levels in BJAB-TACI, ADAM10, and ADAM17 single KO and in double KO cells.

(H) BJAB-TACI and TACI-negative BJAB cells were incubated for 3 hr with 100 ng/mL BAFF trimer or BAFF 60-mer and analyzed for ADAM10 and ADAM17 surface expression by flow cytometry. The expression levels of at least three independent experiments were normalized to untreated cells. Significant differences were calculated by one-way analysis of variance with Bonferroni's multiple comparison test.

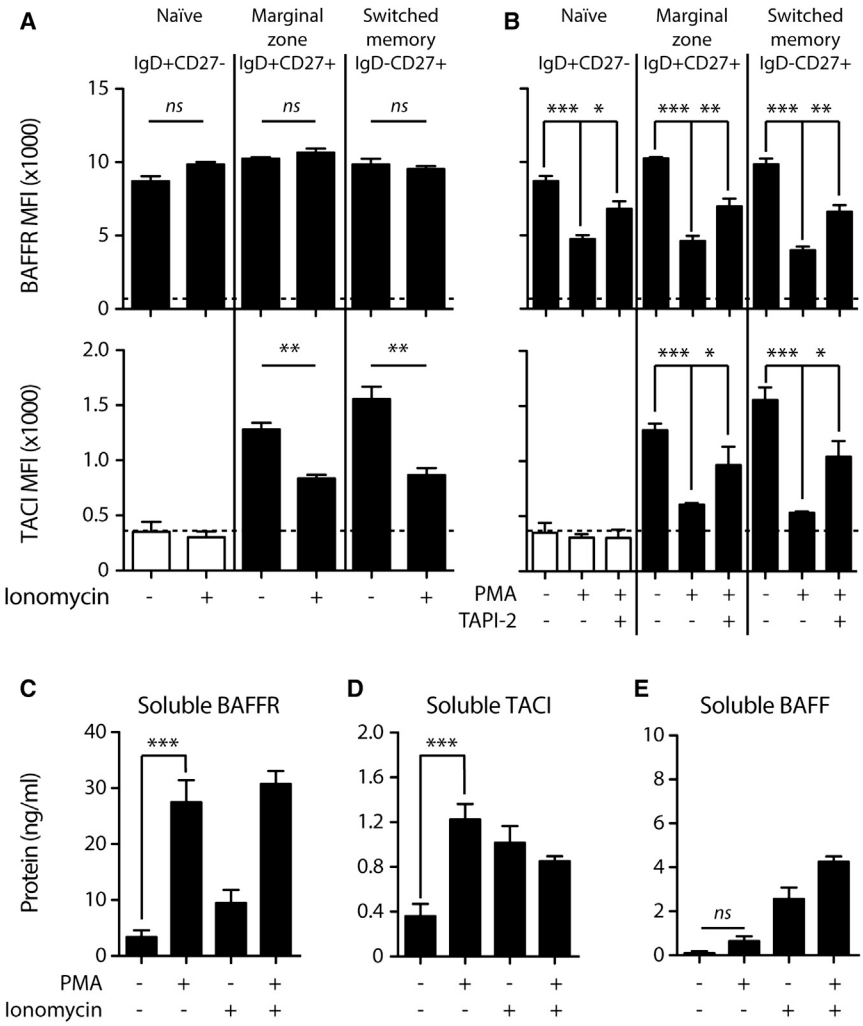

Figure 5. Phorbol Ester Induces ADAM17-Mediated Processing of BAFFR

(A) B cells were activated for 1 hr with 1 μg/mL ionomycin and analyzed for BAFFR and TACI expression by flow cytometry.

(B) B cells were activated for 1 hr with 5 ng/mL PMA in presence or absence of 4 μM of the ADAM17 inhibitor TAPI-2 and analyzed for BAFFR expression by flow cytometry. Plots represent the mean values and SEM of the median of fluorescence intensity (MFI) from three independent experiments. The gating strategy is outlined in Figure S1.

(C) Detection of soluble BAFFR by ELISA in supernatants of PBMC treated overnight with 5 ng/mL PMA, 1 μg/mL ionomycin, or the combination of both.

(D) Same as (C) but for detection of soluble TACI.

(E) Same as (C) but for detection of soluble BAFF. Plots represent mean values ± SEM from at least two independent experiments carried out in quadruplicates. Differences were calculated by one-way analysis of variance with Bonferroni's multiple comparison test.

Vincent et al., 2014). We therefore assumed that BAFF binding to BAFFR would induce the processing of BAFFR expressed by GC B cells.

Immunofluorescence analysis of human tonsil sections showed high BAFFR levels in CD38$^-$ Ki67$^-$ CXCR4$^-$ mantle zone (MZ) B cells outside the GC. In contrast, CXCR4$^+$ Ki67$^-$ cells of the GC LZ express intermediate levels of BAFFR whereas Ki67$^+$ cells of the DZ express very low levels of BAFFR (Figure 7A). To gain insight into the expression levels of BAFFR in DZ and LZ B cells we analyzed surface and total BAFFR expression levels by flow cytometry and western blot (Figures 7B, 7C, and S6). Using B cells from human tonsils, we found that GC B cells from the DZ expressed no or little BAFFR, whereas LZ and mantle zone B cells expressed normal levels (Figures 7B, 7C, and S6). Even after removing BAFFR-bound BAFF by acid elution, DZ B cells still expressed less BAFFR than LZ B cells (Figures 7D and S6B). To test whether this difference correlates with BAFFR processing, tonsillar B cells were cultivated with or without metalloprotease inhibitors in the presence or absence of BAFF (Figure 7B, western blots). Addition of BAFF induces BAFFR processing in switched memory and LZ B cells, which was inhibited by the pan-metalloprotease inhibitor marimastat. In contrast to LZ B cells, DZ B cells expressed low amounts of BAFFR; treatment with BAFF did not induce processing but the addition of marimastat slightly increased total BAFFR protein levels (Figure 7B, western blots). Inhibition of ADAM10 by GI254023x in DZ B cells slightly enhanced BAFFR levels. As the inhibition of ADAM17 by TAPI-2 or by marimastat led to a 2-fold increase in BAFFR MFI (Figure 7E), BAFFR in DZ B cells seem to be cleaved mainly by ADAM17. Moreover, BAFFR surface expression levels slowly increased when the cells were cultivated for 3 days, while the addition of TAPI-2 or marimastat promoted an almost complete recovery within 1 day (Figures 7F and S6C). Therefore, BAFFR levels in DZ B cells seem to be regulated by ADAM17 activity.

These results show that BAFFR expressed by GC B cells of the DZ is loaded with BAFF. Combined to the cellular activation of GC B cells, BAFF binding to BAFFR induces ADAM17-dependent degradation of BAFFR, which represent a mechanism to control BAFFR surface levels on germinal center B cells.

DISCUSSION

Receptor-ligand interactions play a central role in regulating cellular homeostasis and development. Therefore, proteolytic processing of ligands and their transmembrane receptors has a direct influence on cellular responses initiated by receptor-dependent signals. In the immune system, ADAM proteases catalyze ectodomain shedding and regulate intramembrane proteolysis of receptors and ligands that are critical for innate and

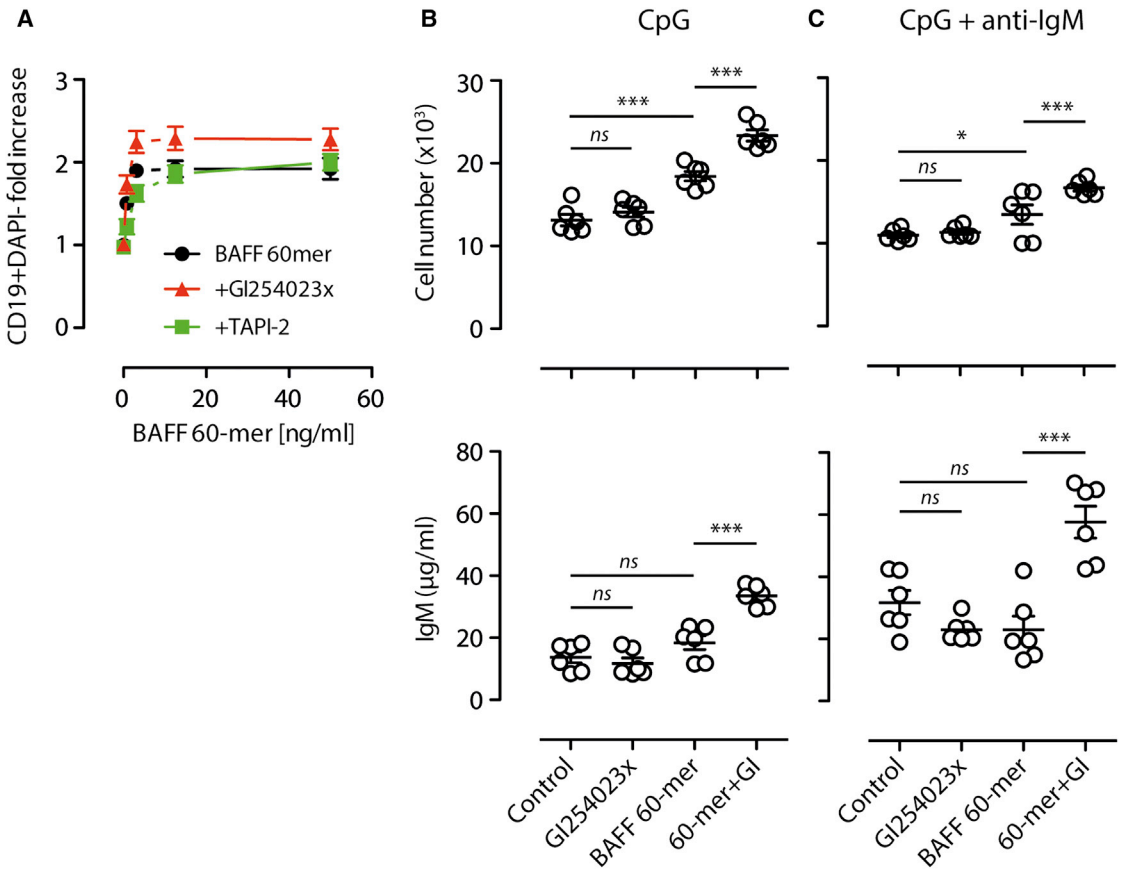

Figure 6. BAFFR Processing Regulates BAFF-Induced B Cell Survival

(A) Pure B cells were cultivated for 3 days in the presence of BAFF 60-mer alone or in combination with 4 μM GI254023x (ADAM10 inhibitor) or 4 μM TAPI-2 (ADAM17 inhibitor). N-fold increase in survival was calculated as [cell number in the presence of BAFF ± inhibitor/cell number w/o BAFF ± inhibitor]. The plot shows the mean and SEM of the fold increase of $CD19^{+}DAPI^{-}$ cells of three independent experiments. Statistically significant differences (** for GI254023x treatment compared to BAFF 60-mer alone) were determined using one-way analysis of variance with Bonferroni's multiple comparison test.

(B) Survival (top) and IgM secretion (bottom) of B cells activated with 0.1 μM CpG in the presence or absence of GI254023x (4 μM) and 25 ng/mL BAFF 60-mer. Cells were analyzed by flow cytometry using timed acquisition. The plot shows cell numbers and means of six independent experiments.

(C) Same as in (B) but for B cells activated with anti-IgM (0.1 μg/mL) and CpG (0.1 μM). Significant differences were calculated by one-way analysis of variance with Bonferroni's multiple comparison test.

adaptive immune responses, including TNF, TNF-receptors, CD40, Notch1, Notch2, CD44, and CD23 (Gibb et al., 2011).

Here, we show that BAFF binding induces the proteolytic cleavage of BAFFR by ADAM10 and/or ADAM17 in TACI-expressing B cells. BAFFR processing is a tightly controlled event, which differs from the previously described constitutive shedding of the closely related receptors TACI and BCMA (Hoffmann et al., 2015; Laurent et al., 2015). Regulated by the degree of BAFF oligomerization, by the co-expression of TACI and by the differential expression of ADAM proteins in B cell subsets, ligand-dependent BAFFR processing appears to be a mechanism to control BAFFR surface expression and BAFF-dependent B cells responses.

In circulating primary human B cells, ADAM10 seems to be responsible for BAFF-induced BAFFR cleavage whereas ADAM17 process BAFFR in DZ germinal center B cells. In Burkitt's lymphoma and in EBV-immortalized B cell lines, the degree of BAFF multimerization was found to regulate the protease involved in BAFFR processing. Binding of BAFF 60-mers activated not only ADAM10 but also induced an increase in the surface levels of ADAM17 leading to activation-induced processing of BAFFR and TACI. This observation is in line with previous reports demonstrating that BAFF trimers preferentially induce BAFFR signaling whereas BAFF 60-mers activate both BAFFR and TACI (Bossen et al., 2008).

Processing of adhesion molecules, receptors, and cytokines by ADAM17 plays an important role in inflammation and autoimmunity (Scheller et al., 2011). Therefore, the activity of the protease is tightly regulated but it can be induced by potent activators such as PMA (Doedens and Black, 2000; Le Gall et al., 2010; Scheller et al., 2011). Using the PKC inhibitor Go6983, the ADAM17-specific inhibitor TAPI-2, and ADAM17 KO cells, we show that the PKC-dependent activation of ADAM17 leads to BAFFR processing in primary human B cells and in B cell lines. In contrast, treatment with ionomycin, which is an inducer of ADAM10 activity (Hundhausen et al., 2007), failed to initiate

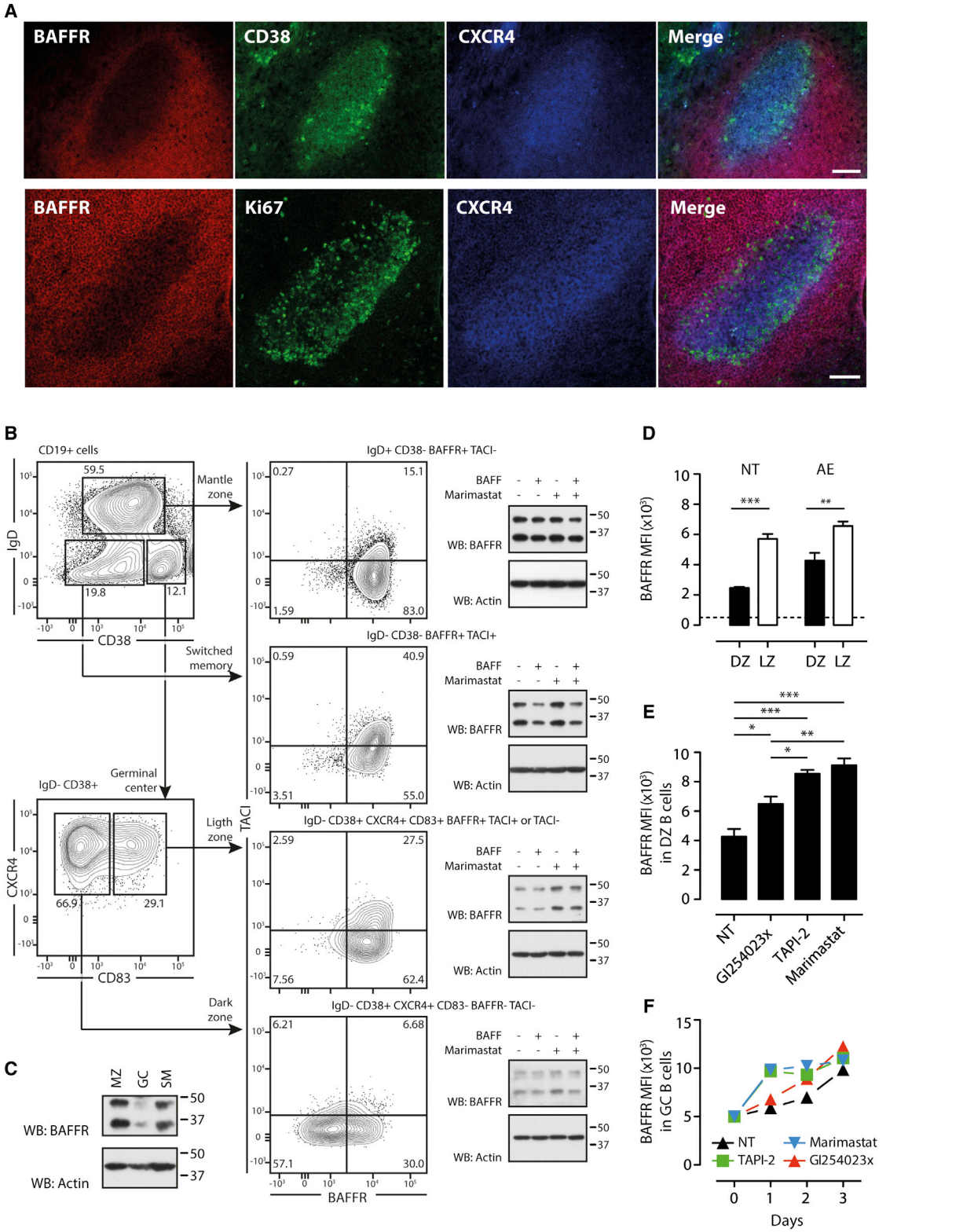

Figure 7. BAFF-Induced BAFFR Processing in B Cells from the Dark Zone of Germinal Centers

(A) Tonsillar cryosections stained with anti-BAFFR (red), anti-CD38 or anti-Ki67 (green), anti-CXCR4 (blue). Mantle zone B cells (BAFFR^{+} CD38^{-} Ki67^{-} CXCR4^{-}) were located around the germinal centers. Germinal center light zone B cells (BAFFR^{low} CXCR4^{+} Ki67^{-}) and dark zone B cells (BAFFR^{low} Ki67^{+}) were identified inside the follicles. Scale bar, 100 μm.

(legend continued on next page)

BAFFR processing in the absence of ligand binding. This suggests, that physiological changes in ADAM17 activity can lead to BAFFR processing and, in consequence, modulate BAFFR function. The decrease in BAFFR and TACI surface levels induced by PMA or ionomycin correlated with increased soluble receptors in supernatants. However, the precise cleavage site of BAFFR by ADAMs still needs to be identified. The membrane-bound C-terminal fragment, which is a product of BAFFR processing, suggests that the cleavage occurs within the extracellular domain. Resistance of BAFFR-Fc to processing leaves a stretch of <10 amino acids within the membrane-proximal part of BAFFR as a target for ADAM10 and/or ADAM17 cleavage. It starts at residue 74 with the sequence N-LPGLL and shares partial homology to a related sequence of TACI (LPGLK) containing the ADAM10 cleavage site (Hoffmann et al., 2015). However, we cannot exclude that processing of BAFFR occurs elsewhere in the extracellular domain.

Similar to what we observed with BAFFR and TACI, it has been previously shown (Urra et al., 2007) that p75 (NGFR, CD271, or TNFRSF16) undergoes ligand-induced shedding in a mechanism requiring the high affinity NGF receptor TrkA. Furthermore, the C-terminal fragment of p75 is internalized and processed by γ-secretase in endosomes, indicating that different TNF receptor family members might share similar processing mechanism following ligand binding.

Surprisingly, TACI turned out to be an essential "co-factor" for BAFF-induced BAFFR processing. Because BAFFR was not processed in EBV cells expressing a CVID-associated TACI variant that cannot bind BAFF, the role of TACI was not just limited to its co-expression. BAFFR cleavage also required a normal degree of BAFFR multimerization and BAFF binding as the multimerization-defective P21R BAFFR variant (Pieper et al., 2014) was not processed following BAFF treatment. Therefore, while TACI is cleaved regardless of BAFFR expression, BAFFR processing required the binding of BAFF to both BAFFR and TACI. The mechanism by which co-expression of TACI allows BAFFR processing has not been completely elucidated. However, proximity ligation assay (PLA) showed a close proximity between TACI and ADAM10 in TACI-positive primary human B cells (Figure S7), which is rather expected for a constitutively shed substrate (Figures 1 and 4) (Hoffmann et al., 2015). Under the same conditions, it is not possible to detect proximity between BAFFR and ADAM10, neither in TACI-negative (CD27-negative) nor in TACI-positive (CD27-positive) B cells. Addition of BAFF induces a positive signal between BAFFR and ADAM10 in TACI-positive (CD27-positive) B cells, suggesting that shared binding of BAFF to BAFFR and TACI might lead to close proximity between BAFFR and ADAM10, thus allowing cleavage of BAFFR (Figure S7). Without BAFF binding, BAFFR would not get in in contact with ADAM10 and might not undergo structural changes that expose its processing site to ADAM proteases.

Similar to CVID patients with complete TACI-deficiency (Castigli et al., 2005; Salzer et al., 2005, 2009), TACI KO mice were reported to have elevated numbers of B cells in the periphery (Seshasayee et al., 2003) accompanied with impaired T-independent humoral responses (Yan et al., 2001; von Bülow et al., 2001). These phenotypes led to the conclusion that TACI may have both activating and inhibitory functions (Lee et al., 2008). Our data and a recent report on constitutive TACI processing (Hoffmann et al., 2015) extend our current view on TACI function by providing insight into TACI-controlled B cell homeostasis. Because TACI is released as a soluble decoy receptor, its expression and processing controls the availability of circulating BAFF and APRIL (Hoffmann et al., 2015). In addition, TACI is also required for BAFF-induced BAFFR processing and this event regulates the pro-survival activity of BAFFR in response to BAFF. Because *TACI* mutations impairing ligand binding and decoy receptor function increase BAFF levels in CVID patients (Kreuzaler et al., 2012; Romberg et al., 2013), they also enhance BAFFR-induced pro-survival signals without being controlled by TACI-dependent BAFFR processing. This scenario would initiate a self-supporting process resulting in the accumulation of B cells, which is supported by our results showing that the inhibition of BAFFR processing by inhibiting ADAM10 increased in a BAFF-dependent manner the number of B cells stimulated in vitro with CpG or anti-IgM and CpG.

Analyzing germinal centers from human tonsils, we found that most of the BAFFRs expressed by DZ (IgD^- $CD38^+$ $CXCR4^+$ $CD83^-$) GC B cells were occupied by BAFF, whereas BAFFRs on LZ (IgD^- $CD38^+$ $CXCR4^{+/low}$ $CD83^+$) GC B cells carried much less BAFF. Because BAFF binding to BAFFR and BAFF-independent activation induce processing of BAFFR by ADAM17, exposure of B cells entering the DZ of GCs to BAFF would elicit BAFFR-induced survival signals that are modulated by ligand- and by activation-induced ADAM17-dependent BAFFR processing. Returning to the LZ, GC B cells seem to express BAFF receptors, which are not or less engaged in BAFF binding. Thus, differential expression of BAFF between the dark and the light zones combined with BAFFR signaling and processing by DZ but not by LZ B cells might contribute to the selection of high affinity switched memory B cells and plasma cells. In fact, T-follicular helper cells seem to be an important source for

(B) Mantle zone ($CD19^+$ IgD^+ $CD38^-$), switched memory ($CD19^+$ IgD^- $CD38^-$), germinal center ($CD19^+$ IgD^- $CD38^+$), dark zone ($CD19^+$ IgD^- $CD38^-$ $CXCR4^+$ $CD83^-$), and light zone ($CD19^+$ IgD^- $CD38^-$ $CXCR4^+$ $CD83^-$) B cells from human tonsils were analyzed for BAFFR and TACI surface expression by flow cytometry. Sorted subsets were incubated overnight in the presence and absence of BAFF and of the pan-metalloprotease inhibitor marimastat. BAFFR expression was analyzed by western blot. One representative experiment out of four is shown. Figure S6 displays the gating strategy.

(C) BAFFR levels by western blot of whole cell lysates of sorted germinal center (GC), mantle zone (MZ), and switched memory (SM) B cells.

(D) Ex vivo tonsillar cells were either analyzed directly or after a 1 min acid (pH 2.4) pulse for BAFFR surface expression by flow cytometry in dark zone (DZ) and light zone (LZ) B cells.

(E) Cells were incubated over night with or without the metalloprotease inhibitors GI254023x, TAPI-2, marimastat (all at 4 μM) and stained for BAFFR expression prior acid elution. NT, non-treated. Mean values and SEM of BAFFR MFI of four tonsil samples are shown. Significant differences were calculated by one-way analysis of variance with Bonferroni's multiple comparison test.

(F) Mean of fluorescence (MFI) of BAFFR expressed by IgD^- $CD38^+$ germinal center B cells from human tonsil treated over 3 days with the indicated metalloprotease inhibitors (all at 4 μM). Acidic elution was performed prior antibody staining. Mean values and SEM of triplicates from one tonsil sample are shown.

BAFF in GCs and the lack of BAFF expression by these cells seems to prevent the selection of high affinity antibody-secreting cells (Goenka et al., 2014). Conversely, it is well established that the negative selection of autoreactive B cells is impaired in transgenic mice overexpressing BAFF (Ota et al., 2010) enhancing the development of autoimmunity and lupus-like symptoms (Mackay et al., 1999; Stohl et al., 2005). The finding, that high BAFF levels in systemic lupus erythematosus (SLE) patients correlate with low BAFFR expression levels on B cells (Zhao et al., 2010) may be explained by our observation that BAFF receptors cannot be stained with specific antibodies while they are binding to BAFF.

In conclusion, BAFF-induced cleavage of BAFFR by ADAM10 and ADAM17 in TACI-expressing B cells is a regulatory mechanism of BAFFR-dependent B cell survival and activation. ADAM10 and ADAM17 play an important role as regulators of lymphocyte development, autoimmunity, inflammation, and tissue regeneration (Gibb et al., 2011; Scheller et al., 2011). According to our data, both proteases also regulate BAFFR-dependent B cell survival and activation. Therefore, the combined targeting of BAFF levels and ADAM activity may be used to modulate B cell development and responses in diseases characterized by disturbed B cell function and ADAM activity.

EXPERIMENTAL PROCEDURES

BAFFR Processing

The work with human material was approved by the Ethics Committee of the Albert-Ludwigs-University Freiburg through approval 169/13. Primary human B cells or cell lines were plated at 1 × 10^6 cells/mL and treated for the indicated time points or overnight (~16 hr) with the indicated doses of ligands. For western blot detection, cells were collected in 1.5 mL tubes, centrifuged at 10,000 rpm for 2 min, the supernatant was eliminated and the pellet resuspended in Laemmli buffer 1×. Samples were vortexed, heated for 5 min at 95°C, and sonicated. For flow cytometry detection, cells were stripped in acid. Briefly, cells were washed twice (256 × *g*; 5 min; room temperature [RT]) with PBS. Pellets were re-suspended in 50 μL citrate buffer (0.1 M citric acid + 0.2 M Na_2HPO_4) pH2.4 and incubated at RT for 1 min. PBS (150 μL) were added and cells were pelleted by centrifugation (256 × *g*; 5 min; 4°C). After two more washing steps (256 × *g*; 5 min; 4°C) in 200 μl FACS buffer, cells were stained with the corresponding antibodies. Flow cytometry analysis was performed using a FACS Canto II (BD Biosciences).

qPCR was performed as described previously (Kienzler et al., 2013; Kraus et al., 2014) using the primer/probe real-time PCR assays for BAFFR (TNFRSF13C) (HS00606874-81) and TACI (TNFRSF13B) (HS00963364-m1) from (Applied Biosystems). PCR fragments were amplified for 2 min at 50°C, 10 min at 95°C followed by 45 cycles consisting of 15 s at 95°C and 1 min at 58°C. Relative expression was calculated using the 22DCq method with cDNA concentrations standardized to the reference gene RPLPO (Applied Biosystems).

In Vitro B Cell Experiments

B cell survival assays were performed as described before (Warnatz et al., 2009; Pieper et al., 2014). B cells (5 × 10^4) were cultivated for 1–3 days in 96-well plates in 200 μL IMDM 10% FCS in the presence or absence of ADAMs inhibitors, BAFF, 0.1 μM CpG, or 0.1 μg/mL goat anti-human IgM $F(ab)_2$. Cells were then analyzed by flow cytometry by timed acquisition.

Immunofluorescence

Cryostat sections (10 μm) of human tonsils were stained as described before (Sic et al., 2014) using anti-human BAFFR PE, anti-Ki67-biotin, anti-CD38 PE (Biolegend), anti-CXCR4 BV421 (Biolegend), and Alexa405-conjugated streptavidin (Thermo Fisher). Optimal antibody concentrations were determined by titration.

Human B Cell Purification

B cells from blood samples were isolated and separated by magnetic cell sorting as described before (Pieper et al., 2014; Sic et al., 2014; Warnatz et al., 2009).

Statistics

Paired t test was applied in Figures 1B, 2I, and 7D. One-way analysis of variance with Bonferroni's multiple comparison test was applied in Figures 4H, 5, 6, and 7E using GraphPad Prism version 5.0a for Macintosh (GraphPad Software; http://www.graphpad.com) (not significant [ns] $p > 0.05$; *$p = 0.01–0.05$; **$p = 0.001–0.01$; ***$p < 0.001$).

Additional information is provided in the Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.02.005.

AUTHOR CONTRIBUTIONS

Conceptualization, C.R.S., P.S., and H.E.; Methodology, C.R.S., P.S., and H.E.; Investigation, C.R.S., P.K., L.M.S., H.S.S., A.K.E., and L.W.; Writing – Original Draft, C.R.S. and H.E.; Writing – Review & Editing, C.R.S., P.S., and H.E.; Funding Acquisition, P.S. and H.E.; Resources, M.S., H.H., U.S., A.G.R., and M.R.; Supervision, C.R.S. and H.E.

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REFERENCES

Bacchelli, C., Buckland, K.F., Buckridge, S., Salzer, U., Schneider, P., Thrasher, A.J., and Gaspar, H.B. (2011). The C76R transmembrane activator and calcium modulator cyclophilin ligand interactor mutation disrupts antibody production and B-cell homeostasis in heterozygous and homozygous mice. J Allergy Clin. Immunol. *127*, 1253–1259.

Bossen, C., and Schneider, P. (2006). BAFF, APRIL and their receptors: structure, function and signaling. Semin. Immunol. *18*, 263–275.

Bossen, C., Cachero, T.G., Tardivel, A., Ingold, K., Willen, L., Dobles, M., Scott, M.L., Maquelin, A., Belnoue, E., Siegrist, C.A., et al. (2008). TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts. Blood *111*, 1004–1012.

Cachero, T.G., Schwartz, I.M., Qian, F., Day, E.S., Bossen, C., Ingold, K., Tardivel, A., Krushinskie, D., Eldredge, J., Silvian, L., et al. (2006). Formation of virus-like clusters is an intrinsic property of the tumor necrosis factor family member BAFF (B cell activating factor). Biochemistry *45*, 2006–2013.

Castigli, E., Wilson, S.A., Garibyan, L., Rachid, R., Bonilla, F., Schneider, L., and Geha, R.S. (2005). TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat. Genet. *37*, 829–834.

Dillon, S.R., Gross, J.A., Ansell, S.M., and Novak, A.J. (2006). An APRIL to remember: novel TNF ligands as therapeutic targets. Nat. Rev. Drug Discov. *5*, 235–246.

Doedens, J.R., and Black, R.A. (2000). Stimulation-induced down-regulation of tumor necrosis factor-alpha converting enzyme. J. Biol. Chem. *275*, 14598–14607.

Figgett, W.A., Fairfax, K., Vincent, F.B., Le Page, M.A., Katik, I., Deliyanti, D., Quah, P.S., Verma, P., Grumont, R., Gerondakis, S., et al. (2013). The TACI receptor regulates T-cell-independent marginal zone B cell responses through innate activation-induced cell death. Immunity *39*, 573–583.

Fortier, M.H., Caron, E., Hardy, M.P., Voisin, G., Lemieux, S., Perreault, C., and Thibault, P. (2008). The MHC class I peptide repertoire is molded by the transcriptome. J. Exp. Med. *205*, 595–610.

Gibb, D.R., Saleem, S.J., Chaimowitz, N.S., Mathews, J., and Conrad, D.H. (2011). The emergence of ADAM10 as a regulator of lymphocyte development and autoimmunity. Mol. Immunol. *48*, 1319–1327.

Goenka, R., Matthews, A.H., Zhang, B., O'Neill, P.J., Scholz, J.L., Migone, T.S., Leonard, W.J., Stohl, W., Hershberg, U., and Cancro, M.P. (2014). Local BLyS production by T follicular cells mediates retention of high affinity B cells during affinity maturation. J. Exp. Med. *211*, 45–56.

Groom, J.R., Fletcher, C.A., Walters, S.N., Grey, S.T., Watt, S.V., Sweet, M.J., Smyth, M.J., Mackay, C.R., and Mackay, F. (2007). BAFF and MyD88 signals promote a lupuslike disease independent of T cells. J. Exp. Med. *204*, 1959–1971.

Gross, J.A., Dillon, S.R., Mudri, S., Johnston, J., Littau, A., Roque, R., Rixon, M., Schou, O., Foley, K.P., Haugen, H., et al. (2001). TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. Immunity *15*, 289–302.

Hoffmann, F.S., Kuhn, P.H., Laurent, S.A., Hauck, S.M., Berer, K., Wendlinger, S.A., Krumbholz, M., Khademi, M., Olsson, T., Dreyling, M., et al. (2015). The immunoregulator soluble TACI is released by ADAM10 and reflects B cell activation in autoimmunity. J. Immunol. *194*, 542–552.

Hundhausen, C., Schulte, A., Schulz, B., Andrzejewski, M.G., Schwarz, N., von Hundelshausen, P., Winter, U., Paliga, K., Reiss, K., Saftig, P., et al. (2007). Regulated shedding of transmembrane chemokines by the disintegrin and metalloproteinase 10 facilitates detachment of adherent leukocytes. J. Immunol. *178*, 8064–8072.

Kalled, S.L. (2002). BAFF: a novel therapeutic target for autoimmunity. Curr. Opin. Investig. Drugs *3*, 1005–1010.

Katsenelson, N., Kanswal, S., Puig, M., Mostowski, H., Verthelyi, D., and Akkoyunlu, M. (2007). Synthetic CpG oligodeoxynucleotides augment BAFF- and APRIL-mediated immunoglobulin secretion. Eur. J. Immunol. *37*, 1785–1795.

Kienzler, A.K., Rizzi, M., Reith, M., Nutt, S.L., and Eibel, H. (2013). Inhibition of human B-cell development into plasmablasts by histone deacetylase inhibitor valproic acid. J. Allergy Clin. Immunol. *131*, 1695–1699.

Kraus, H., Kaiser, S., Aumann, K., Bönelt, P., Salzer, U., Vestweber, D., Erlacher, M., Kunze, M., Burger, M., Pieper, K., et al. (2014). A feeder-free differentiation system identifies autonomously proliferating B cell precursors in human bone marrow. J. Immunol. *192*, 1044–1054.

Kreuzaler, M., Rauch, M., Salzer, U., Birmelin, J., Rizzi, M., Grimbacher, B., Plebani, A., Lougaris, V., Quinti, I., Thon, V., et al. (2012). Soluble BAFF levels inversely correlate with peripheral B cell numbers and the expression of BAFF receptors. J. Immunol. *188*, 497–503.

Laurent, S.A., Hoffmann, F.S., Kuhn, P.H., Cheng, Q., Chu, Y., Schmidt-Supprian, M., Hauck, S.M., Schuh, E., Krumbholz, M., Rübsamen, H., et al. (2015). γ-Secretase directly sheds the survival receptor BCMA from plasma cells. Nat. Commun. *6*, 7333.

Le Gall, S.M., Maretzky, T., Issuree, P.D., Niu, X.D., Reiss, K., Saftig, P., Khokha, R., Lundell, D., and Blobel, C.P. (2010). ADAM17 is regulated by a rapid and reversible mechanism that controls access to its catalytic site. J. Cell Sci. *123*, 3913–3922.

Lee, J.J., Ozcan, E., Rauter, I., and Geha, R.S. (2008). Transmembrane activator and calcium-modulator and cyclophilin ligand interactor mutations in common variable immunodeficiency. Curr. Opin. Allergy Clin. Immunol. *8*, 520–526.

Mackay, F., and Schneider, P. (2009). Cracking the BAFF code. Nat. Rev. Immunol. *9*, 491–502.

Mackay, F., Woodcock, S.A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., Tschopp, J., and Browning, J.L. (1999). Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J. Exp. Med. *190*, 1697–1710.

Mantchev, G.T., Cortesão, C.S., Rebrovich, M., Cascalho, M., and Bram, R.J. (2007). TACI is required for efficient plasma cell differentiation in response to T-independent type 2 antigens. J. Immunol. *179*, 2282–2288.

McHeyzer-Williams, M., Okitsu, S., Wang, N., and McHeyzer-Williams, L. (2011). Molecular programming of B cell memory. Nat. Rev. Immunol. *12*, 24–34.

Ng, L.G., Mackay, C.R., and Mackay, F. (2005). The BAFF/APRIL system: life beyond B lymphocytes. Mol. Immunol. *42*, 763–772.

Ota, M., Duong, B.H., Torkamani, A., Doyle, C.M., Gavin, A.L., Ota, T., and Nemazee, D. (2010). Regulation of the B cell receptor repertoire and self-reactivity by BAFF. J. Immunol. *185*, 4128–4136.

Pieper, K., Grimbacher, B., and Eibel, H. (2013). B-cell biology and development. J. Allergy Clin. Immunol. *131*, 959–971.

Pieper, K., Rizzi, M., Speletas, M., Smulski, C.R., Sic, H., Kraus, H., Salzer, U., Fiala, G.J., Schamel, W.W., Lougaris, V., et al. (2014). A common single nucleotide polymorphism impairs B-cell activating factor receptor's multimerization, contributing to common variable immunodeficiency. J. Allergy Clin. Immunol. *133*, 1222–1225.

Purcell, A.W. (2004). Isolation and characterization of naturally processed MHC-bound peptides from the surface of antigen-presenting cells. Methods Mol. Biol. *251*, 291–306.

Romberg, N., Chamberlain, N., Saadoun, D., Gentile, M., Kinnunen, T., Ng, Y.S., Virdee, M., Menard, L., Cantaert, T., Morbach, H., et al. (2013). CVID-associated TACI mutations affect autoreactive B cell selection and activation. J. Clin. Invest. *123*, 4283–4293.

Salzer, U., Chapel, H.M., Webster, A.D., Pan-Hammarström, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H.H., Rockstroh, J.K., Schneider, P., Schäffer, A.A., et al. (2005). Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat. Genet. *37*, 820–828.

Salzer, U., Bacchelli, C., Buckridge, S., Pan-Hammarström, Q., Jennings, S., Lougaris, V., Bergbreiter, A., Hagena, T., Birmelin, J., Plebani, A., et al. (2009). Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes. Blood *113*, 1967–1976.

Scheller, J., Chalaris, A., Garbers, C., and Rose-John, S. (2011). ADAM17: a molecular switch to control inflammation and tissue regeneration. Trends Immunol. *32*, 380–387.

Schiemann, B., Gommerman, J.L., Vora, K., Cachero, T.G., Shulga-Morskaya, S., Dobles, M., Frew, E., and Scott, M.L. (2001). An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science *293*, 2111–2114.

Schuepbach-Mallepell, S., Das, D., Willen, L., Vigolo, M., Tardivel, A., Lebon, L., Kowalczyk-Quintas, C., Nys, J., Smulski, C., Zheng, T.S., et al. (2015). Stoichiometry of heteromeric BAFF and APRIL cytokines dictates their receptor binding and signaling properties. J. Biol. Chem. *290*, 16330–16342.

Seshasayee, D., Valdez, P., Yan, M., Dixit, V.M., Tumas, D., and Grewal, I.S. (2003). Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. Immunity *18*, 279–288.

Shulga-Morskaya, S., Dobles, M., Walsh, M.E., Ng, L.G., MacKay, F., Rao, S.P., Kalled, S.L., and Scott, M.L. (2004). B cell-activating factor belonging to the TNF family acts through separate receptors to support B cell survival and T cell-independent antibody formation. J. Immunol. *173*, 2331–2341.

Sic, H., Kraus, H., Madl, J., Flittner, K.A., von Münchow, A.L., Pieper, K., Rizzi, M., Kienzler, A.K., Ayata, K., Rauer, S., et al. (2014). Sphingosine-1-phosphate receptors control B-cell migration through signaling components associated with primary immunodeficiencies, chronic lymphocytic leukemia, and multiple sclerosis. J. Allergy Clin. Immunol. *134*, 420–428.

Stohl, W., Xu, D., Kim, K.S., Koss, M.N., Jorgensen, T.N., Deocharan, B., Metzger, T.E., Bixler, S.A., Hong, Y.S., Ambrose, C.M., et al. (2005). BAFF overexpression and accelerated glomerular disease in mice with an incomplete genetic predisposition to systemic lupus erythematosus. Arthritis Rheum. *52*, 2080–2091.

Stohl, W., Hiepe, F., Latinis, K.M., Thomas, M., Scheinberg, M.A., Clarke, A., Aranow, C., Wellborne, F.R., Abud-Mendoza, C., Hough, D.R., et al.; BLISS-52 Study Group; BLISS-76 Study Group (2012). Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. Arthritis Rheum. *64*, 2328–2337.

Suzuki, K., Maruya, M., Kawamoto, S., Sitnik, K., Kitamura, H., Agace, W.W., and Fagarasan, S. (2010). The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. Immunity *33*, 71–83.

Tak, P.P., Thurlings, R.M., Rossier, C., Nestorov, I., Dimic, A., Mircetic, V., Rischmueller, M., Nasonov, E., Shmidt, E., Emery, P., and Munafo, A. (2008). Atacicept in patients with rheumatoid arthritis: results of a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating, single- and repeated-dose study. Arthritis Rheum. *58*, 61–72.

Treml, L.S., Carlesso, G., Hoek, K.L., Stadanlick, J.E., Kambayashi, T., Bram, R.J., Cancro, M.P., and Khan, W.N. (2007). TLR stimulation modifies BLyS receptor expression in follicular and marginal zone B cells. J. Immunol. *178*, 7531–7539.

Urra, S., Escudero, C.A., Ramos, P., Lisbona, F., Allende, E., Covarrubias, P., Parraguez, J.I., Zampieri, N., Chao, M.V., Annaert, W., and Bronfman, F.C. (2007). TrkA receptor activation by nerve growth factor induces shedding of the p75 neurotrophin receptor followed by endosomal gamma-secretase-mediated release of the p75 intracellular domain. J. Biol. Chem. *282*, 7606–7615.

Vincent, F.B., Morand, E.F., Schneider, P., and Mackay, F. (2014). The BAFF/APRIL system in SLE pathogenesis. Nat. Rev. Rheumatol. *10*, 365–373.

von Bülow, G.U., van Deursen, J.M., and Bram, R.J. (2001). Regulation of the T-independent humoral response by TACI. Immunity *14*, 573–582.

Wallace, D.J., Stohl, W., Furie, R.A., Lisse, J.R., McKay, J.D., Merrill, J.T., Petri, M.A., Ginzler, E.M., Chatham, W.W., McCune, W.J., et al. (2009). A phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus. Arthritis Rheum. *61*, 1168–1178.

Warnatz, K., Salzer, U., Rizzi, M., Fischer, B., Gutenberger, S., Böhm, J., Kienzler, A.K., Pan-Hammarström, Q., Hammarström, L., Rakhmanov, M., et al. (2009). B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. Proc. Natl. Acad. Sci. USA *106*, 13945–13950.

Yan, M., Wang, H., Chan, B., Roose-Girma, M., Erickson, S., Baker, T., Tumas, D., Grewal, I.S., and Dixit, V.M. (2001). Activation and accumulation of B cells in TACI-deficient mice. Nat. Immunol. *2*, 638–643.

Zhao, L.D., Li, Y., Smith, M.F., Jr., Wang, J.S., Zhang, W., Tang, F.L., Tian, X.P., Wang, H.Y., Zhang, F.C., Ba, D.N., et al. (2010). Expressions of BAFF/BAFF receptors and their correlation with disease activity in Chinese SLE patients. Lupus *19*, 1534–1549.