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The Immunoregulator Soluble TACI Is Released by ADAM10 and Reflects B Cell Activation in Autoimmunity


BAFF and a proliferation-inducing ligand (APRIL), which control B cell homeostasis, are therapeutic targets in autoimmune diseases. TACI-Fc (atacicept), a soluble fusion protein containing the extracellular domain of the BAFF–APRIL receptor TACI, was applied in clinical trials. However, disease activity in multiple sclerosis unexpectedly increased, whereas in systemic lupus erythematosus, atacicept was beneficial. In this study, we show that an endogenous soluble TACI (sTACI) exists in vivo. TACI proteolysis involved shedding by a disintegrin and metalloproteinase 10 releasing sTACI from activated B cells. The membrane-bound stub was subsequently cleaved by γ-secretase reducing ligand-independent signaling of the remaining C-terminal fragment. The shed ectodomain assembled ligand independently in a homotypic way. It functioned as a decoy receptor inhibiting BAFF- and APRIL-mediated B cell survival and NF-κB activation. We determined sTACI levels in autoimmune diseases with established hyperactivation of the BAFF–APRIL system. sTACI levels were elevated both in the cerebrospinal fluid of the brain-restricted autoimmune disease multiple sclerosis correlating with intrathecal IgG production, as well as in the serum of the systemic autoimmune disease systemic lupus erythematosus correlating with disease activity. Together, we show that TACI is sequentially processed by a disintegrin and metalloproteinase 10 and γ-secretase. The released sTACI is an immunoregulator that shares decoy functions with atacicept. It reflects systemic and compartmentalized B cell accumulation and activation.

B cells play a significant role in the pathogenesis of autoimmunity and B cell modulating therapies are promising in the treatment of a variety of autoimmune diseases (1). Regulation of B cell homeostasis involves the BAFF–APRIL system that is comprised of two ligands, BAFF and a proliferation-inducing ligand (APRIL), and three receptors, B cell maturation Ag (BCMA), transmembrane activator and CAML interactor (TACI), and BAFF receptor (2).

In systemic lupus erythematosus (SLE), an involvement of the BAFF–APRIL axis is prominent, as mice overexpressing BAFF develop an SLE-like phenotype, BAFF is elevated in the serum of SLE patients, and the mAb belimumab targeting BAFF is beneficial in a proportion of SLE patients (3, 4). In multiple sclerosis (MS), an organ-specific autoimmune disease characterized by local Ig production with long-term persistence of B cells in the CNS (5, 6), BAFF is upregulated in MS plaques and is produced by astrocytes (7). Although depletion of B cells in MS with anti-CD20 Abs is promising (8), targeting the B cell survival factors BAFF and APRIL with atacicept, a recombinant fusion protein containing the extracellular ligand-binding portion of TACI linked to the Fc domain of human IgG, unexpectedly increased disease activity in MS patients (9), whereas in SLE atacicept was beneficial at least at a high dose (10). TACI is a type I–oriented transmembrane protein belonging to the TNFR superfamily. It is expressed on CD27+ memory B cells, F.S.H. performed experiments, was involved in study design, and wrote the paper; P.-H.K, S.A.L., S.M.H., and K.B. performed experiments; M. Krumholz was involved in study design; M. Khademi, T.O., M.D., H.W.P., T.A., E.H., and T.K. provided clinical samples; H.C.C. provided ADAM10 conditional knockout mice; H.W., R.H., S.F.L., and E.M. designed the study and wrote the paper. All authors discussed results and commented on the manuscript.

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Abbreviations used in this article: ADAM, a disintegrin and metalloproteinase; APRIL, a proliferation-inducing ligand; BCMA, B cell maturation Ag; CIS, clinically isolated syndrome; CRD, cysteine-rich domain; CSF, cerebrospinal fluid; CTI, C-terminal fragment; IP, immunoprecipitation; MS, multiple sclerosis; NB, neuroborreliosis; NP-40, Nonidet P-40; OND, other neurologic disease; PVDF, polyvinylidene difluoride; RR-MS, relapsing-remitting multiple sclerosis; shRNA, short hairpin RNA; SLE, systemic lupus erythematosus; SP-MS, secondary progressive multiple sclerosis; STACI, soluble TACI; TACI, transmembrane activator and CAML interactor; TACI-ΔEcto, TACI lacking the ectodomain; TACI-FL, full-length human TACI; TACI-N-FLAG, TACI with an N-terminal FLAG tag; TACI-N-ΔHA, TACI with an N-terminal hemagglutinin tag.

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plasma cells and a subpopulation of CD27+ B cells and is induced early upon B cell activation (11). The ectodomain of TACI contains two cysteine-rich domains (CRDs). The first CRD is involved in ligand-independent assembly of TACI into multimeric complexes, whereas the second CRD is required for binding of BAFF and APRIL (12). Ligand binding to TACI recruits signaling molecules to the intracellular domain of TACI, which leads to activation of NFAT and NF-kB (13, 14). Studies of TACI−/− mice showed that this receptor is both a positive and negative regulator of B cell responses (15–17). Mutations in TACI are a cause of common variable immunodeficiency and IgA deficiency (18, 19). However, some of these patients in addition develop signs of autoimmunity and lymphoproliferation (19).

Importantly, the functions of some transmembrane receptors extend beyond signal transmission, as they can be processed into soluble receptors (20). In this regard, proteases of the α-disintegrin and metalloproteinase (ADAM) family are involved in ectodomain shedding of a variety of membrane proteins (21). This can modulate signaling activity either by downregulation of membrane-bound receptors or by the release of soluble receptors like soluble TNFR1 (22) or soluble IL-6R (23). In the case of type I transmembrane proteins, the γ-secretase complex may further cleave the remaining fragment within the plasma membrane (24) in a process called regulated intramembrane proteolysis (25).

In this study, we show that the TACI extracellular domain is shed from activated B cells by ADAM10, giving rise to soluble TACI (sTACI). The remaining C-terminal fragment (CTF) is cleaved by γ-secretase. sTACI assembles homotypically; it binds BAFF and APRIL to block NF-kB activation and B cell survival. In systemic (SLE) and compartmentalized (MS) immunopathologies, we detected elevated levels of sTACI establishing sTACI as a potential biomarker.

Materials and Methods

Patients

All patient samples were collected following written informed consent according to local ethics policy guidelines in Stockholm, Berlin, and Munich and the Declaration of Helsinki. We analyzed the following samples: cerebrospinal fluid (CSF) from 37 untreated MS patients (clinically isolated syndrome [CIS]; n = 10; relapsing-remitting multiple sclerosis [RR-MS]; n = 20; and secondary progressive multiple sclerosis [SP-MS]; n = 7) and from 20 untreated patients with other neurologic diseases (ONDs) (sensory symptoms: n = 1; depression and idiopathic pain: n = 1; alcohol-related spastic paraparesis: n = 1; and secondary progressive multiple sclerosis (SP-MS): n = 1); plasma from 57 untreated patients with ONDs: cerebrospinal fluid (CSF) from 33 healthy volunteers. Intrathecal IgG production was calculated as: IgG-index (CSF IgG/CSF albumin)/(serum IgG/serum albumin). Production was calculated as: IgG-index (CSF IgG/CSF albumin)/(serum IgG/serum albumin).

Cell culture

Raji cells were cultured in RPMI 1640 (Sigma-Aldrich)/10% FBS (Biochrom AG)/100 U/ml penicillin plus 100 μg/ml streptomycin (Life Technologies, Invitrogen)/1% nonessential amino acids (Life Technologies, Invitrogen)/1 mM sodium pyruvate (Life Technologies, Invitrogen)/2 mM l-glutamine (Pan Biotech). HEK293T cells were cultured in DMEM (Sigma-Aldrich)/10% FBS/100 U/ml penicillin plus 100 μg/ml streptomycin.

Transient transfection

HEK293T cells were transfected using Lipofectamine 2000 (Invitrogen). sTACI containing and control supernatants were generated by transfection of 10 μg TACI in pCMV-XL4 or an empty vector. After 3 d, supernatants were harvested and centrifuged two times at 400 × g for 7 min and one time at 20,000 × g for 30 min.

Human B cell culture and stimulation

PBMCs were isolated by density gradient separation using Pancoll (Pan Biotech). Human B cells were isolated by negative selection using the EasySep Human B Cell Enrichment Kit (StemCell Technologies). Human B cells were seeded at 8 × 10^5 cells/ml and activated using ODN 2006 (2.5 μg/ml; Invitrogen), anti-IL-2 (10 μg/ml; Jackson ImmunoResearch Laboratories), R848 (1 μg/ml; Sigma-Aldrich), recombinant human IL-2 (25 ng/ml; R&D Systems), coculture with CD40L expressing mouse L cells (5 × 10^3 cells/ml), and recombinant human IL-21 (50 ng/ml; Biocytence). Supernatants were collected after 4 d. sTACI production and IgG production were measured. TACI surface expression was determined using a PE mouse anti-human TACI Ab (5 μg/ml; clone Fab1741P; R&D Systems) and the corresponding PE mouse IgG1 isotype control (5 μg/ml; clone X40; BD Biosciences).

Murine B cell culture and survival assay

For murine B cell cultivation, spleens from C57BL/6 mice (8–12 wk of age) were passed over a 40-μm cell strainer. Red cell lysis was performed using ACK buffer (150 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA) for 2 min on ice. B cells were isolated using the EasySep Mouse B Cell Isolation Kit (StemCell Technologies) by negative selection. The murine B cells were seeded at a concentration of 3 × 10^5 cells/ml on cell-culture plates coated with anti-murine IgM Ab (2.5 μg/ml) overnight at room temperature. Cells were treated with the indicated concentrations of HEK293T cell supernatants containing sTACI and control supernatants generated as described above. Additionally, TACI-Fc was added (R&D Systems), and cells were stimulated with BAFF (100 ng/ml; R&D Systems) and APRIL (100 ng/ml; AdipoGen). After 2 d of cultivation, mouse B cells were analyzed by flow cytometry using a PE anti-mouse CD19 Ab (1 μg/ml; clone MB19-1; eBioscience) and APC TO-PRO-3 Iodide (250 nM; Life Technologies). PE-positive and allophycocyanin-negative cells were determined.

Western blotting and immunoprecipitation

To analyze the role of γ-secretase, TACI with a C-terminal FLAG-tag was transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen). In control amounts of DAPT were added. After 48 h, cells were lysed in Nonidet P-40 (NP-40) buffer (150 mM NaCl, 50 mM Tris [pH 7.5], 1% NP-40), and complete protease inhibitor mixture; Roche Applied Science). Proteins were electrophoresed on 10–20% Tricine gels, transferred to polyvinylidene difluoride (PVDF) membranes, and blotted with an anti-FLAG Ab (clone M2; Sigma-Aldrich). To control for equal protein loading, membranes were stained with anti-β-actin Ab (clone C4; Santa Cruz Biotechnology). Proteins were immunoprecipitated from TACI from HEK293T, a monoclonal mouse anti-TACI Ab (clone MAB174; R&D Systems) or a mouse IgG1 control (R&D Systems) was used. For IP from Raji cell supernatant, the polyclonal goat anti-TACI Ab (clone AF1741; R&D Systems) or a goat IgG control (R&D Systems) was applied. Abs were coupled to Dynabeads Protein G (Life Technologies) and cross-linked with bis-sulfosuccinimidyl-suberate (Pierce). sTACI derived from HEK293T cells was eluted with NaPAGE LDS Sample Buffer (Life Technologies),electrophoresed on 4–12% Bis-Tris gels (Life Technologies) with MES-running buffer with MES-running buffer, blotted on PVDF membranes, and stained with a monoclonal mouse anti-TACI Ab (clone MAB174; R&D Systems). Alternatively, the PVDF membrane was

Protease inhibitors

DAPT (Merck Calbiochem), TAPI-1 (Merck Calbiochem), C3 (Merck Calbiochem), and GI254023X (kindly provided by Dr. Andreas Ludwig, University of Erlangen, Erlangen, Germany) were used.
cut prior to staining at the height corresponding to stTACI, and N-terminal sequencing was performed. stTACI derived from Raji cells was obtained by acidic elution. For coimmunoprecipitation of TACI-N-HA and TACI-N-FLAG, HEK293T cells were transfected with the respective expression constructs. Forty-eight hours later, cells and supernatants were harvested. Cells were lysed in IP buffer (0.5% NP-40, 50 mM HEPES, 250 mM NaCl, 5 mM EDTA, and complete protease inhibitor mixture; Roche Applied Science). IP was performed using anti-FLAG magnetic beads (Sigma-Aldrich). IP eluates and pre-IP samples were subjected to ELISA.

**Mass spectrometry and Edman sequencing**

stTACI derived from Raji cells was digested in solution with trypsin or GluC; stTACI derived from HEK293T cells was digested with trypsin and chymotrypsin. Mass spectrometry (LTQ Orbitrap XL; Thermo Scientific) was performed.

**ELISA**

Human stTACI and IgG concentrations were determined using the Human TACI DuoSet (DY174; R&D Systems) and the Human IgG ELISA development kit (Maabtech), respectively. Complex formation between stTACI and BAFF and APRIL was determined by coating anti-FLAG Ab (M2; 5 μg/ml; Sigma-Aldrich) on ELISA plates, incubating them with BAFF-FLAG (Enzo Life Sciences) and APRIL-FLAG (Adipogen) (200 ng/ml) and adding stTACI and TACI-Fc (R&D Systems) (25 ng/ml). Bound TACI was detected using the TACI DuoSet ELISA Kit. To detect TACI-N-HA coimmunoprecipitated with TACI-N-FLAG, ELISA plates were coated with an anti-HA.11 (Covance) Ab (5 μg/ml). The following ELISA steps were performed using the TACI DuoSet ELISA Kit (R&D Systems).

**NF-κB reporter assay**

HEK293T cells were cotransfected with a firefly luciferase reporter plasmid, the internal control CMV Renilla luciferase plasmid, and the respective expression plasmids. The decoy-function of stTACI was assessed by adding BAFF or APRIL-FLAG (100 ng/ml) to supernatants containing stTACI or to control supernatants. After incubation at 37°C for 30 min, supernatants were added to the BCMA-transfected cells. Sixteen hours later, cells were lysed with passive lysis buffer (Promega), and reporter gene activity was determined with passive lysis buffer (Promega), and reporter gene activity was determined with Renilla luciferase assay (Promega). The following ELISA steps were performed using the TACI DuoSet ELISA Kit (R&D Systems).

**Retrovirus production and transduction of B cells from ADAM10 conditional knockout mice**

We isolated splenic B cells from ADAM10 conditional knockout mice (26), which contain two flox sites flanking the ADAM10 gene. Mouse B cells were stimulated for 2 d with CpG ODN 1668 (20 μg/ml; Invivogen) and rhTNF-α (25 μg/ml; R&D Systems). Retrovirus was produced as described previously (27) using the vector pMSCV expressing GFP or the CRE-recombinase followed by the sequence of the self-cleaving T2A peptide and GFP. B cells were spin-infected as described previously (27). On day 4, TACI expression was determined using a rat monoclonal anti-TACI Ab (clone FAB1041A; 5 μg/ml; R&D Systems). Knockdown of ADAM10 was determined by Western blot using a rabbit monoclonal anti-ADAM10 Ab (clone EP85622; Abcam) in FACS-sorted GFP-positive cells.

**Lentiviral short hairpin RNA–mediated knockdown**

Lentivirus production was performed as described previously (28). Short hairpin RNA (shRNA) sequences are listed in Supplemental Table 1. Raji cells were plated at 1 × 10⁶ cells/ml and transfected with conditioned medium containing the lentiviruses. The transduced Raji cells were plated at 5 × 10⁶ cells/ml. Twenty-four hours later, supernatants were harvested and analyzed for stTACI concentration by ELISA. TACI surface expression was determined by FACS using a mouse monoclonal anti-TACI Ab (clone MAB174; 1 μg/ml; R&D Systems) and the corresponding mouse IgG1 isotype control (R&D Systems).

**Real-time PCR**

RNA from transduced Raji cells was isolated using the RNasy Micro Kit (Qiagen). cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For real-time PCR, TaqMan assays for ADAM9 and ADAM17 (Applied Biosystems) and PrimeTime qPCR Assays for ADAM9 and ADAM19 (Integrated DNA Technologies) were used in combination with the TaqMan PCR Core Reagent Kit (Applied Biosystems). Cyclophilin (Applied Biosystems) expression was determined as a housekeeping gene. Samples were run in MicroAmp Optical 96-well reaction plates (Applied Biosystems) in a 7900HT Fast Real-Time PCR System (Applied Biosystems). Data was analyzed using SDSv2.3 software (Applied Biosystems).

**Statistics**

Statistical significance was assessed with Prism Software (GraphPad) by unpaired or paired, nonparametric, or parametric t test analysis or by Spearman correlation, where appropriate. The p values *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 were considered significant and designated accordingly.

**Results**

stTACI is released by activated B cells

As TACI is described as an ambivalent regulator of B cell homeostasis, we speculated that this effect could be due to a soluble variant of TACI. Therefore, we stimulated primary human B cells with a panel of different B cell activators and determined plasma membrane expression of TACI (Fig. 1A) and in parallel release of stTACI (Fig 1B). Although B cells in human blood expressed little TACI on their surface, we noted a strong induction by TLR7/8 and TLR9 agonists and by CD40L, which was further enhanced by addition of IL-21 (Fig 1A) (11). stTACI release and TACI surface expression, induced by the different stimuli, correlated strongly (Fig. 1C), suggesting that stTACI production by primary B cells parallels levels of TACI on the cell surface. In addition, we analyzed whether stTACI release is linked to differentiation into Ig-secreting cells. We found that activated B cells released stTACI without producing IgG (e.g., after CD40L and R848 stimulation) (Fig. 1B). To identify stimuli that trigger stTACI release from membrane-bound TACI, we stimulated the Burkitt lymphoma cell line Raji, which endogenously expresses TACI, with different TLR ligands and cytokines (polyinosinic-polycytidylic acid, CpG ODN 2006, TNF-α, IFN-γ, and PMA). We did not observe a strong modulation of stTACI release (Supplemental Fig. 1A), and only PMA weakly and transiently induced TACI shedding (Supplemental Fig. 1B). Thus, when TACI is present on the cell surface, it is shed without the need of an additional stimulus. Together, this suggests that stTACI release reflects TACI expression on the cell surface.

stTACI represents the extracellular part of TACI

Next, we determined the m.w. and amino acid composition of stTACI. We immunoprecipitated stTACI from the supernatant of HEK293T cells that had been transfected with full-length human TACI. stTACI had a molecular mass of ~13 kDa in Western blot analysis and silver staining (Fig. 2A). For mass spectrometry, we analyzed the band detected by silver staining and stTACI immunoprecipitated from Raji cell supernatant. After trypsin, chymotrypsin, and GluC digestion as well as N-terminal sequencing, we found soluble TACI to be composed of the extracellular part of TACI (Fig. 2B). The sequence at the C terminus ended with K154, adjacent to L155 in TACI. stTACI had a molecular mass of ~13 kDa in Western blot analysis and silver staining (Fig. 2A). For mass spectrometry, we analyzed the band detected by silver staining and stTACI immunoprecipitated from Raji cell supernatant. After trypsin, chymotrypsin, and GluC digestion as well as N-terminal sequencing, we found soluble TACI to be composed of the extracellular part of TACI (Fig. 2B). The sequence at the C terminus ended with K154, adjacent to L155 in TACI. stTACI was derived from HEK293T cells and Raji cells (Fig. 2B). K154/L155 is within the juxtamembrane region and comprises a typical cleavage motif of metalloproteinases of the ADAM and membrane-type matrix metalloproteinase family. Therefore, K154/L155 could be the naturally occurring cleavage site of TACI resulting in generation of stTACI. Alternatively, this site can also be recognized by trypsin.

ADAM10 sheds TACI

To identify the protease that sheds TACI, we applied different protease inhibitors to Raji cells. We found that the metalloproteinase inhibitor TAPI-I, as well as GI254023X (29), a specific ADAM10 inhibitor, blocked stTACI release and increased surface expression of TACI.
FIGURE 1. sTACI is released by activated B cells and closely correlates with membrane-bound TACI. (A and B) Human purified B cells were activated using CD40L, IL-21, CD40L + IL-21, CpG (ODN2006), anti-IgM, anti-IgM + IL-21, R848, IL-2, and R848 + IL-2 for 4 d. (A) TACI surface expression was determined by FACS 4 d after activation. Filled histograms represent the isotype control; solid lines the TACI expression. (B) sTACI and IgG production were analyzed by ELISA. (C) Mean fluorescence intensity (MFI) of TACI surface expression was calculated by subtracting the isotype fluorescence signal. sTACI production and MFI of membrane-bound TACI correlated strongly ($p = 0.0105$, $r = 0.7818$ Spearman correlation). Representative data of two different donors.
ADAM10 RELEASES SOLUBLE TACI FROM B CELLS

Supplemental Fig. 2B and also when GFP-positive cells were transfected with full-length TACI or an empty vector as control (IP: MAB174) followed by SDS-PAGE and Western blotting (WB). IP eluates were probed for TACI (MAB174). Silver staining was performed after IP from supernatant of HEK293T cells transfected with CRE-T2A-GFP virus (Supplemental Fig. 2C–E). This shows that knockdown of ADAM10 led to a decrease of sTACI from Raji cell supernatant followed by acidic elution and digestion with trypsin or chymotrypsin. IP of sTACI from Raji cell supernatant was excised and digested with trypsin or chymotrypsin. The amino acid sequences of TACI and peptides identified by mass spectrometry after tryptic (green), chymotryptic (blue), or GluC (orange) digestion are shown. sTACI derived from HEK293T cells was additionally analyzed by N-terminal sequencing (Edman) (red).

**FIGURE 2.** sTACI is composed of the extracellular part of TACI (A) sTACI was immunoprecipitated from supernatant of HEK293T cells transfected with TACI-FL or an empty vector as control (IP: MAB174) followed by SDS-PAGE and Western blotting (WB). IP eluates were probed for TACI (MAB174). Silver staining was performed after IP from supernatant of HEK293T cells transfected with full-length TACI or an empty vector as control (IP: AF174). (B) After IP of sTACI from HEK293T cell supernatant, the band corresponding to sTACI in silver staining was excised and digested with trypsin or chymotrypsin. IP of sTACI from Raji cell supernatant followed by acidic elution and digestion with trypsin or GluC was performed. The amino acid sequences of TACI and peptides identified by mass spectrometry after tryptic (green), chymotryptic (blue), or GluC (orange) digestion are shown. sTACI derived from HEK293T cells was additionally analyzed by N-terminal sequencing (Edman) (red).

(Fig. 3A, 3B). The β-secretase inhibitor C3 (30) and the γ-secretase inhibitor DAPT had no effect (Fig. 3A, 3B). A combined treatment with TAPI-1 and GI254023X did not further decrease sTACI production (Fig. 3A), suggesting that besides ADAM10 no other pro tease susceptible to inhibition by TAPI-1 is involved in TACI cleavage. TAPI-1 also reduced the release of sTACI from primary human B cells activated with CD40L and IL-21, whereas DAPT had no effect (Supplemental Fig. 1C). To confirm the role of TAPI-1 in TACI cleavage, we lentivirally transduced Raji cells with shRNAs targeting ADAM9, ADAM10, ADAM17, and ADAM19 (Fig. 3C, 3D). As knockdown of ADAM17 influenced cell survival and proliferation, we corrected sTACI levels for cell number. Knockdown was confirmed by quantitative PCR (Fig. 3E). We found that only a knockdown of ADAM10 led to a decrease of sTACI production (Fig. 3D), paralleled by an increase of TACI surface expression (Fig. 3C). None of the other applied shRNAs influenced membrane-bound TACI or sTACI production (Fig. 3C, 3D). The effect mediated by ADAM10 shRNA was similar to that of TAPI-1 in ELISA and FACS (Fig. 3A, 3B). To confirm the role of ADAM10 in TACI shedding in primary B cells, we used murine B cells from conditional ADAM10 KO mice that contain two flox sites flanking the ADAM10 gene (26). To achieve the ADAM10 knockout, B cells were transduced with retroviral particles expressing the CRE recombinase followed by the self-cleaving T2A peptide (31) and GFP (CRE-T2A-GFP), which resulted in efficient knockout of ADAM10 as confirmed by Western blot (Supplemental Fig. 2A). As a control, a retrovirus expressing GFP was used. TACI expression was determined by FACS. Knockout of ADAM10 led to an increase of TACI surface expression when comparing cells transduced with the control virus to cells transduced with the CRE-T2A-GFP virus (Supplemental Fig. 2B) and also when GFP-positive cells were compared with GFP-negative cells after transduction with the CRE-T2A-GFP virus (Supplemental Fig. 2C–E). This shows that ADAM10 also sheds TACI from primary murine B cells.

**γ-Secretase cleaves the CTF of TACI**

As ectodomain shedding of type I membrane proteins is commonly followed by γ-secretase cleavage of the CTF (25), we investigated whether γ-secretase is also involved in the cleavage of the TACI CTF. When we transfected TACI with a C-terminal FLAG-tag in HEK293T cells and treated them with DAPT, we observed accumulation of the TACI CTF (Fig. 4A), which demonstrates that the CTF of TACI is cleaved by γ-secretase. As a next step, we investigated potential functional consequences of TACI cleavage by γ-secretase. We used an NF-κB reporter assay in HEK293T cells transfected with either full-length human TACI (TACI-FL) or TACI-Δ-Ecto resembling the TACI CTF. Both TACI-FL and TACI-Δ-Ecto exhibited constitutive NF-κB activation in the absence of a ligand (Fig. 4B). Spontaneous dimerization via the CRD1 (12) is believed to confer constitutive activity to TACI. TACI-Δ-Ecto lacks the CRD1. The CTF might therefore dimerize independent of the CRD1 due to high amounts of TACI present in this overexpression system. Additionally, we stimulated the transfected cells with BAFF. As expected, BAFF increased NF-κB activation in cells transfected with TACI-FL, but did not activate TACI-Δ-Ecto, which lacks the ligand binding domain (Fig. 4B). DAPT treatment increased NF-κB signaling mediated by TACI-Δ-Ecto, indicating that cleavage of the CTF of TACI by γ-secretase reduces NF-κB activation. This suggests that γ-secretase cleavage of TACI CTF might be relevant to limit NF-κB activation after TACI ectodomain shedding. DAPT treatment had no effect on cells transfected with TACI-FL presumably because the amount of TACI cleaved by ADAM10 compared with TACI-FL was too low.
sTACI assembles in homotypic interaction

To establish whether sTACI exists as a monomeric or an oligomeric form, we coexpressed TACI–N-HA or TACI–N-FLAG in HEK293T cells. We found that sTACI–N-HA could be coimmunoprecipitated with sTACI–N-FLAG, which argues for a homotypic interaction of sTACI monomers in the absence of ligand (Fig. 5A). As a positive control, full-length TACI–N-HA derived from cell lysates was coimmunoprecipitated with full-length TACI–N-FLAG, because membrane-bound TACI was reported previously to interact homotypically (12) (Fig. 5B).

sTACI binds BAFF and APRIL, blocking B cell survival and NF-κB activation

To explore the function of sTACI generated after ADAM10 cleavage, we determined binding of sTACI to BAFF and APRIL. We established an ELISA in which sTACI was captured by BAFF-FLAG or APRIL-FLAG immobilized on an anti-FLAG–coated ELISA plate. We found that sTACI as well as TACI-Fc bound both BAFF and APRIL (Fig. 6A). In the following, we evaluated the functional consequences of this binding and determined the influence of sTACI on BCMA-mediated NF-κB activation in the presence of BAFF and APRIL.

**FIGURE 3.** ADAM10 sheds TACI. (A and B) Raji cells were treated for 24 h with the indicated concentrations of DAPT, C3, TAPI-1, GI354023X, and TAPI-1 + GI354023X. (A) sTACI release was determined by ELISA (combined data of four independent experiments [mean ± SEM]). (B) TACI surface expression was analyzed by FACS. Only the high inhibitor concentrations with corresponding vehicle control and isotype controls are depicted (representative data of four independent experiments). (C-E) Raji cells were lentivirally transduced with two different shRNAs targeting ADAM9, ADAM10, ADAM17, and ADAM19, respectively, or a nontargeting shRNA (Con). For comparison, cells were treated with TAPI-1. (C) TACI surface expression was analyzed by FACS; representative data of two independent experiments. (D) sTACI release was determined by ELISA and controlled for cell number (two-tailed, paired t test, including two technical replicates of the respective experiments); combined data of two independent experiments (mean ± SEM). (E) Knockdown of ADAM9, ADAM10, ADAM17, and ADAM19 was determined by quantitative PCR; 1 and 2 represent two different shRNAs targeting the same ADAM; combined data of two independent experiments (mean ± SEM). ***p < 0.01, ****p < 0.001.
APRIL in HEK293T cells. In our assay, transfection of BCMA leads to lower constitutive activity and higher activation after stimulation compared with TACI transfection and was therefore more suitable for testing a possible decoy function of sTACI. Addition of sTACI to APRIL and BAFF-stimulated cells dose-dependently blocked NF-κB activation (Fig. 6B). BAFF-mediated NF-κB activation could be blocked slightly more efficiently (Fig. 6B). Recombinant TACI-Fc was used as a control and exhibited comparable blocking capacity as sTACI (Fig. 6B). To further support the functional relevance of sTACI, we tested the impact of sTACI.
on survival of primary B cells. We purified mouse B cells from spleens and stimulated them with anti-IgM plus BAFF or APRIL and increasing concentrations of sTACI. BAFF increased B cell survival more strongly than APRIL. This enhanced survival by both ligands could efficiently be blocked by sTACI and TACI-Fc (Fig. 6C). These data point to a negative regulatory function of sTACI on B cell survival.

sTACI as a potential biomarker in human immunopathologies

We evaluated whether sTACI is detectable in: 1) inflammatory CNS diseases with compartmentalized IgG production (MS and neuroborreliosis [NB]); and 2) a systemic autoimmune disease (SLE). We found significantly increased sTACI levels in the cerebrospinal fluid (CSF) of MS patients compared with patients with ONDs without signs of CNS inflammation (Fig. 7A). Moreover, sTACI levels correlated strongly with intrathecal IgG production (Fig. 7B). This correlation was confirmed in a second cohort of 25 MS patients ($p < 0.0001; r = 0.82$). We investigated whether this elevation is specific for MS or a consequence of compartmentalized inflammation in the brain. Therefore, we analyzed patients with NB, which is like MS characterized by intrathecal IgG production. We detected elevation of sTACI and correlation to intrathecal IgG production also in NB, which suggests that increased sTACI levels in the CSF are not disease specific, but the consequence of local accumulation and activation of B cells. In contrast to CSF, in plasma, no significant difference between patients with MS and ONDs was detected (Supplemental Fig. 3A). sTACI levels in CSF did not differ among CIS, RR-MS, and SP-MS patients (Supplemental Fig. 3B). Immunosuppressive treatment

FIGURE 6. sTACI acts as a decoy for BAFF and APRIL and blocks B cell survival. (A) ELISA plates were coated with anti-FLAG Abs (M2; 5 μg/ml); BAFF-FLAG and APRIL-FLAG were added; and sTACI (25 ng/ml) derived from supernatant of transfected HEK293T cells or concentrated supernatants of Raji cells and TACI-Fc (25 ng/ml) were used; combined data of three independent experiments (mean ± SEM). (B) HEK293T cells were transfected with full-length BCMA (2.5 ng) and a luciferase-based NF-κB reporter. BAFF and APRIL (100 ng/ml) were added together with increasing amounts of sTACI-containing supernatant (33, 100, and 300 ng/ml), control supernatant, or TACI-Fc (300 ng/ml), and NF-κB activation was determined (two-tailed, paired t test); combined data of three independent experiments (mean ± SEM). (C) Murine B cells were activated via anti-IgM and cultured for 2 d with APRIL or BAFF (100 ng/ml) in the presence or absence of sTACI (100, 200, and 400 ng/ml) and TACI-Fc (400 ng/ml). Survival of B cells was determined by FACS analyzing the percentage of living CD19+ cells after staining with TO-PRO-3 iodide. Viability was calculated in relation to the BAFF- and APRIL-induced survival that was assigned as 1 (two-tailed, paired t test between BAFF-treated and BAFF + sTACI/TACI-Fc–treated conditions); combined data of four to five independent experiments (mean ± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. 

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influenced sTACI levels, as we found decreased sTACI levels in the CSF of MS patients 12 mo after initiation of monthly i.v. natalizumab treatment (Fig. 7C). Furthermore, sTACI levels were transiently reduced 3 d after corticosteroid treatment and returned to baseline levels within a period of 4 wk (Supplemental Fig. 3C). In SLE, we found significantly increased sTACI levels in untreated patients compared with healthy control patients (Fig. 7D), which decreased upon treatment (Fig. 7D). Interestingly, sTACI levels in untreated patients correlated with disease activity as expressed by the SLE Disease Activity Index (Fig. 7E).

Discussion
In this study, we report that the membrane-bound BAFF–APRIL receptor TACI undergoes ectodomain shedding by ADAM10 and consecutive cleavage by γ-secretase. Shedding of TACI was a consequence of membrane expression of TACI on activated B cells. The generation of soluble receptors is a common principle involving a variety of membrane proteins like growth factors, receptors and their ligands, cytokines, and cell adhesion molecules (20, 21). We identified ADAM10 as the sheddase releasing sTACI, whereas the prototype of this superfamily, TNFR1, is shed by ADAM17 (32). ADAM10 is believed to be mainly involved in the constitutive shedding of membrane proteins, whereas ADAM17 can be activated by PMA (33). Consistent with this principle, we found ADAM10-dependent sTACI production to mainly reflect TACI expression on the cell surface and observed only little induction of shedding by PMA.

TACI shedding was followed by γ-secretase cleavage of the remaining membrane stub representing the CTF. It is unclear whether cleavage of TACI by γ-secretase solely leads to the degradation of the TACI CTF (proteasome of the membrane) (34) or to the generation of a TACI intracellular domain with signaling function as is the case for a few other described γ-secretase substrates [e.g., Notch (35)]. Inhibition of γ-secretase increased ligand-independent NF-κB activation mediated by TACI–Δ-Ecto in transfected HEK293T cells. We speculate that cleavage of the TACI CTF by γ-secretase also takes place in primary B cells, thereby restricting TACI-mediated NF-κB activation after shedding of the ectodomain. However, formal proof for this hypothesis is lacking at the moment.

Previous work with TACI−/− mice and common variable immunodeficiency patients revealed a dual role of TACI in B cell homeostasis (15–19). On the one hand, TACI promotes IgG and IgA class-switch recombination (18, 36), maintains Ab production (18, 19), and increases survival of malignant B cells (37). On the other hand, TACI has negative regulatory effects on B cells, because TACI-deficient mice show a high number of hyperreactive B cells resulting in autoimmunity and lymphoma development (15–17). Mechanisms that might contribute to this negative regulatory effect include: 1) TACI-mediated induction of BLIMP-1, which orchestrates the switch from B cell proliferation to plasma

FIGURE 7. sTACI as a biomarker. (A–E) sTACI levels were determined by ELISA in CSF or serum; horizontal bars indicate the mean. (A) sTACI is elevated in the CSF of patients with CIS/MS and NB compared with ONDs (two-tailed, unpaired, and nonparametric t test). (B) sTACI in the CSF correlates with intrathecal IgG production (MS/CIS: p < 0.0001; r = 0.75; NB: p = 0.057; r = 0.9429; OND: NS, Spearman correlation); some data points of OND patients are hidden behind data points of CIS/MS and NB patients. (C) sTACI levels in CSF decrease after 12 mo of natalizumab treatment (n = 25) (two-tailed, paired t test). (D) sTACI is elevated in the serum of untreated SLE patients and less prominent also in the treated SLE cohort compared with healthy controls (HC; two-tailed, unpaired, and nonparametric t test); with treatment, sTACI levels decrease compared with the untreated SLE patients (two-tailed, unpaired, and nonparametric t test). (E) sTACI in serum of untreated SLE patients correlates strongly with disease activity quantified by the SLE Disease Activity Index (SLEDAI; p = 0.0019; r = 0.697, Spearman correlation). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
cell differentiation (38); 2) involvement of TACI in activation-induced cell death of marginal zone B cells (39); and 3) the role of TACI in central removal of autoreactive B cells (40). We propose that the release of endogenous sTACI contributes to these negative regulatory features of TACI, because the decoy functions of sTACI reduce BAFF- and APRIL-mediated survival of different B cell subpopulations.

We found sTACI to interact homotypically, analogously to membrane-bound TACI that preassembles as an oligomeric complex prior to ligand binding (12). Oligomerization increases the binding avidity for ligands, so that the oligomeric structure of sTACI suggests a functionally relevant decoy activity. The fusion protein TACI-Fc (atacicept), which is used in clinical trials, is dimerized via its Fc domain (41). We compared endogenous sTACI with the pharmacological agent TACI-Fc in ELISAs analyzing the binding of BAFF and APRIL, in NF-κB reporter assays and in survival assays with primary B cells. These experiments showed that endogenous sTACI and TACI-Fc share essential decoy functions. TACI-Fc (atacicept) reduced Ig levels and mature B cell counts in clinical studies (9). All of this suggests that endogenous sTACI is a similar negative regulator of the B cell compartment in vivo. We propose that an equilibrium of sTACI and BAFF controls B cell numbers fitting to the observation of raised BAFF levels and increased survival of naive B cells in TACI−/− mice (15, 16).

We analyzed sTACI levels in patients with systemic and compartmentalized systemic autoimmune diseases. Our data indicate that sTACI is produced by locally or systemically accumulating activated B cells and plasma cells. In SLE, sTACI was elevated in serum. This can readily be explained by a hyperactivation of the B cell compartment and increased levels of circulating plasma cells in SLE (3, 42). Interestingly, in SLE, we observed a close correlation of sTACI levels with disease activity. This suggests that sTACI might serve as a useful biomarker (e.g., for individualizing [and thereby optimizing] B cell–targeting therapies). In a phase II clinical trial investigating the effects of belimumab in SLE patients, patients with high concentrations of anti-nuclear Abs and anti-dsDNA Abs responded better to treatment (43). However, baseline BAFF values did not correlate with treatment response (44). Whether sTACI helps to identify patients who are likely to benefit from belimumab or other B cell–directed therapies remains to be studied. In MS, sTACI was not increased in blood, but elevated in CSF, in which levels correlated with intrathecal Ig production. Natalizumab treatment decreased sTACI levels in the CSF, fitting the concept to natalizumab prevents entry of VLA-4+ lymphocytes into the CNS, including sTACI levels in the CSF, fitting to the concept that natalizumab treatment response (44). Whether sTACI helps to identify patients with anti-nuclear Abs and anti-dsDNA Abs responded better to treatment (44). In a phase II clinical trial investigating the effects of belimumab in SLE patients, patients with high concentrations of anti-nuclear Abs and anti-dsDNA Abs responded better to treatment (43). However, baseline BAFF values did not correlate with treatment response (44). Whether sTACI helps to identify patients who are likely to benefit from belimumab or other B cell–directed therapies remains to be studied. In MS, sTACI was not increased in blood, but elevated in CSF, in which levels correlated with intrathecal Ig production. Natalizumab treatment decreased sTACI levels in the CSF, fitting the concept to natalizumab prevents entry of VLA-4+ lymphocytes into the CNS, including circulating B cells that express substantial levels of VLA-4 (45). The fact that atacicept increased disease activity in MS patients (9), whereas in SLE, it showed beneficial clinical effects (10), could interfere in the equilibrium between sTACI and BAFF involved in fine-tuning the balance between effector B cells and regulatory B cells.

In summary, the discovery of the existence of sTACI and its effects as a negative immunoregulator extends our understanding of the complexity of the BAFF–APRIL system. Our study unravels the biochemical basis of sTACI generation. Further, we show that sTACI offers potential as a novel biomarker in MS and SLE and may be useful for therapy optimization.

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Disclosures
The authors have no financial conflicts of interest.

References
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