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


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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 subunit 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. The Journal of Immunology, 2015, 194: 000-000.
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 a 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 disorders (ONDs) (sensory symptoms; n = 7; cerebrovascular disease; n = 1; migraine; n = 1; vertigo; n = 1; syringomyelia; n = 1; spinal stenosis; n = 1; neurethasia; n = 1; alcohol-related spastic paraparesis; n = 1; hearing deficit; n = 1; depression and idiopathic pain; n = 1; fatigue; n = 1; bipolar disorder; n = 1; schizophrenia; n = 1; and diplopia; n = 1); plasma from 57 untreated MS patients (CIS; n = 18; RR-MS; n = 23; and SP-MS; n = 16) and 18 untreated patients with ONDs; CSF from 25 MS patients before and after 12 mo after treatment with natalizumab; serum from 17 untreated SLE patients (CIS: n = 10; depression and idiopathic pain: n = 1; migraine: n = 1; vertigo: n = 1; syringomyelia: n = 1; spinal stenosis: n = 1; neurethasia: n = 1; alcohol-related spastic paraparesis: n = 1; hearing deficit: n = 1; depression and idiopathic pain: n = 1; fatigue: n = 1; bipolar disorder: n = 1; schizophrenia: n = 1; and diplopia: n = 1); plasma from 57 untreated MS patients (CIS; n = 18; RR-MS; n = 23; and SP-MS; n = 16) and 18 untreated patients with ONDs; and serum from 33 healthy volunteers.

**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.

**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% 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 twice 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 × 10⁵ cells/ml and activated using ODN2006 (2.5 μg/ml; Invitrogen), anti-IdM (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 × 10⁶ cells/ml), and recombinant human IL-21 (50 ng/ml; eBioscience). 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 M 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 × 10⁶ cells/ml on cell-culture plates coated with anti-murine IgM Abs (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; biocis) and APC TO-PRO-3 Iodide (250 nM; Life Technologies). PE-positive and allopseudocyanin-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 addition, low 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). For immunoprecipitation, 5 × 10⁶ 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 AF174; 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-sulfosuccimidyl-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, blotted on PVDF membranes, and stained with a monoclonal mouse anti-TACI Ab (clone MAB174; R&D Systems). Alternatively, the PVDF membrane was...
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 coinmunoprecipitation 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 (Mabtech), 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 coinmunoprecipitated 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 using firefly luciferase substrate (Biozym) and Renilla luciferase (Promega), respectively. To analyze the role of γ-secretase on NF-κB activation, TACI–Δ-Ekot or full-length TACI was transfected together with the above-described luciferase plasmids. Eight hours after transfection, DAPT and BAFF were added. Sixteen hours later, NF-κB-activation was measured as described.

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 rCD40L 2.5 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-mouse 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 EPR5622; 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 × 10^6 cells/ml and transfected with conditioned medium containing the lentiviruses. The transduced Raji cells were plated at 5 × 10^6 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 RNeasy Micro Kit (Qiagen). cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For real-time PCR, TaqMan assays were used for ADAM10 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, as 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 soluble TACI to be composed of the extracellular part 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.

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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...
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.
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 acetic 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).

(35A, 3B). The β-secretase inhibitor C3 (30) and the γ-secretase inhibitor DAPT had no effect (35A, 3B). A combined treatment with TAPI-1 and GI254023X did not further decrease sTACI production (Fig. 3A), suggesting that besides ADAM10 no other protease 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 ADAM10 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.
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 the CTF of TACI (representative data of three independent experiments). Relative intensities are depicted below the bands. (B) HEK293T cells were transfected with 1 ng of TACI-FL or TACI-Δ-Ecto and a luciferase-based NF-κB reporter. DAPT (1 μM) and BAFF (100 ng/ml) were added as indicated, and NF-κB activation was determined (two-tailed, paired t tests); combined data of five independent experiments (mean ± SEM). *p < 0.05, **p < 0.01.

FIGURE 4. γ-Secretase degrades the CTF of TACI shutting down NF-κB activation. (A) HEK293T cells were transfected with TACI-FL with a C-terminal FLAG-tag and treated with the indicated concentrations of DAPT or vehicle control. After 48 h, lysates were obtained for SDS-PAGE and Western blotting. Lysates were probed for expression of tagged TACI demonstrating TACI-FL and the CTF of TACI (representative data of three independent experiments). Relative intensities are depicted below the bands. (B) HEK293T cells were transfected with 1 ng of TACI-FL or TACI-Δ-Ecto and a luciferase-based NF-κB reporter. DAPT (1 μM) and BAFF (100 ng/ml) were added as indicated, and NF-κB activation was determined (two-tailed, paired t tests); combined data of five independent experiments (mean ± SEM). *p < 0.05, **p < 0.01.

FIGURE 5. sTACI assembles homotypically independent of a ligand. (A and B) HEK293T cells were transfected with expression plasmids containing TACI with an N-terminal FLAG-tag or HA-tag as indicated. Forty-eight hours after transfection, supernatants (A) were harvested, and cell lysates (B) were prepared. A part of the supernatants (A) and lysates (B) prior to IP were stored; the remaining supernatants and lysates were subjected to FLAG-IP, and the IP eluates plus the supernatants and lysates stored prior to IP were measured by ELISA. An anti-HA Ab was used for coating, and an anti-TACI Ab was applied for detection; combined data of three independent experiments (mean ± SEM).
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, 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

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**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.
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...
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 decay 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 decay 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 hyper-activation 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 BAFF-depletion with rituximab in relapsing-remitting multiple sclerosis (9), whereas in SLE, it showed beneficial clinical effects (10), the fact that atacicept increased disease activity in MS patients could interfere in the equilibrium between sTACI and BAFF in vivo. The authors have no financial conflicts of interest.

References


