Brief Definitive Report

TACI Is a TRAF-interacting Receptor for TALL-1, a Tumor Necrosis Factor Family Member Involved in B Cell Regulation

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Abstract

We and others recently reported tumor necrosis factor (TNF) and apoptosis ligand–related leukocyte-expressed ligand 1 (TALL-1) as a novel member of the TNF ligand family that is functionally involved in B cell proliferation. Transgenic mice overexpressing TALL-1 have severe B cell hyperplasia and lupus-like autoimmune disease. Here, we describe expression cloning of a cell surface receptor for TALL-1 from a human Burkitt's lymphoma RAJI cell library. The cloned receptor is identical to the previously reported TNF receptor (TNFR) homologue transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI). Murine TACI was subsequently isolated from the mouse B lymphoma A20 cells. Human and murine TACI share 54% identity overall. Human TACI exhibits high binding affinities to both human and murine TALL-1. Soluble TACI extracellular domain protein specifically blocks TALL-1–mediated B cell proliferation without affecting CD40- or lipopolysaccharide-mediated B cell proliferation in vitro. In addition, when injected into mice, soluble TACI inhibits antibody production to both T cell–dependent and –independent antigens. By yeast two-hybrid screening of a B cell library with TACI intracellular domain, we identified that, like many other TNFR family members, TACI intracellular domain interacts with TNFR-associated factor (TRAF)2, 5, and 6. Correspondingly, TACI activation in a B cell line results in nuclear factor κB and c-Jun NH_2-terminal kinase activation. The identification and characterization of the receptor for TALL-1 provides useful information for the development of a treatment for B cell–mediated autoimmune diseases such as systemic lupus erythematosus.

Key words: TACI • TNFR family • TALL-1 • B cell stimulation • autoimmune disease

Introduction

The TNFR family includes, among others, TNFR1, TNFR2, Fas, CD40, OX40, 4-1BB, death receptor (DR)3/Wsl-1, DR4, DR5, another TNFR-associated factor (TRAF) receptor (ATAR), osteoprotegerin (OPG), and receptor activator of nuclear factor (NF)-κB (RANK [1–9]). These receptors share similar extracellular domain architecture of multiple cysteine-rich repeats, each containing ~40 amino acids with six cysteines (1). The extracellular domains are usually preceded by hydrophobic signal peptides. Soluble receptors could be generated by deleting the transmembrane and intracellular domains. The intracellular domains lack enzymatic activities. The receptors may be divided into two subgroups based on the presence (e.g., TNFR1, Fas, DR3, DR4, DR5) or absence (e.g., TNFR2, CD40, RANK) of death domains within their intracellular domains (10). The receptors generally signal through direct interaction with death domain proteins (e.g., TNFR-associated death domain [TRADD], Fas-associated death do-

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main [FADD], receptor-interacting protein [RIP]) or with TRAF proteins (e.g., TRAF2, TRAF3, TRAF5, and TRAF6), triggering cellular signaling pathways leading to apoptosis, NF-κB activation, and/or c-Jun NH_2-terminal kinase (JNK) activation (10).

We and others recently reported that TNF- and apoptosis ligand–related leukocyte expressed ligand 1 (TALL-1)/B lymphocyte stimulator (BlyS)/B cell activating factor belonging to the TNF family (BAFF)/TNF homologue that activates apoptosis, NF-κB, and JNK (THANK) is a novel member of the TNF ligand family involved in B cell proliferation (11–15). TALL-1 is a potent B cell costimulatory factor, and acts by direct binding and by activating its cell surface receptor on B cells. Transgenic mice overexpressing TALL-1 have severe B cell hyperplasia and hypergammaglobulinemia (11, 16). These mice also developed autoimmune lupus-like disease characterized by the presence of autoantibodies and immune complex deposits in the kidney (11, 16).

Here, we report expression cloning of a TALL-1 receptor from the human Burkitt's lymphoma RAJI cell line. The receptor is identical to transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI), a previously reported TNFR homologue identified through its interaction with CAML (17). Our findings suggest the potential presence of another unique subgroup within the TNFR family.

Materials and Methods

Reagents. RAJI cells and A20 cells (American Type Culture Collection) were maintained in high-glucose RPMI containing 10% FCS, 100 μg/ml penicillin G, and 100 μg/ml streptomycin. A20 cDNA library was prepared using the Superscript Plasmid System (GIBCO BRL). Human lymphocyte matchmaker cDNA library was generated from mRNA of an EBV-transformed peripheral blood B cell population (CLONTECH Laboratories, Inc.). Recombinant TALL-1 protein was generated as described previously (11). TALL-1 Europium labeling was performed with Wallac Delfia reagent according to the manufacturer's suggestions. Fc-tagged TALL-1 protein was generated by the fusing OPG signal peptide followed by human IgG-γ1 Fc in frame to the NH_2-terminus of TALL-1 amino acid 128–285. The protein was expressed in baculovirus and purified with protein A–sepharose column (5). Soluble TACI protein amino acids 1–165 followed by a His_6-tag or human IgG-γ1 Fc were expressed in *Escherichia coli*. After solubilization of the inclusion bodies, the refolded protein was purified by cation exchange chromatography.

Expression Cloning. A RAJI cell expression library was generated by ligating RAJI cDNA into a mammalian expression vector using the Superscript Plasmid System (GIBCO BRL) according to the manufacturer's suggestions. The library was arrayed into segregated pools containing ~100 clones per pool, and the DNA was purified from 1 ml overnight cultures of each pool grown. Plasmid DNA from each culture was prepared using the Qiawell 96 Ultra Plasmid Kit (QIAGEN), following the manufacturer's instructions. Arrayed pools were individually transfected into 293 cells (American Type Culture Collection), then assayed for the presence of Europium-labeled TALL-1 protein binding using a Victor™ plate reader (Wallac, Inc.)

B Cell Proliferation Assay. Purified (10^5) B cells from C57BL/6 (B6) mice (11) were cultured in MEM plus 10% heat-inactivated FCS in triplicate in a 96-well flat-bottomed plate with 10 ng/ml TALL-1 protein, 2 μg/ml goat $F(ab')_2$ anti–mouse IgM (Jackson ImmunoResearch Laboratories), and an indicated amount of recombinant soluble TACI protein for a period of 4 d at 37°C, 5% CO_2. Proliferation was measured by the uptake of radioactive [^{3}H]thymidine in the last 18 h of pulse.

TACI Expression on PBMCs. Human PBMCs from healthy donors were isolated using Ficoll-paque density centrifugation. Cells were washed and incubated with 1 μg/ml anti-CD3 antibody. Expression of TALL-1 receptor on activated CD4/CD8 T cells was detected using Flag-tagged TALL-1 followed by biotinylated anti-Flag antibody and streptavidin-PE. Biotinylated anti-Flag antibody and streptavidin-PE reagents were used as controls for nonspecific staining. FITC-conjugated anti-CD4 or anti-CD8 antibodies (BD PharMingen) were used to detect TALL-1 receptor on specific cell types. Expression of cell surface molecules was determined using CELLQuest™ software by FACS® (Becton Dickinson).

Induction and Detection of Anti-KLH and Anti-Pneumovax Antibodies. Mice (9–11-wk-old Balb/c females; Charles River Laboratories) were immunized on day 0 subcutaneously with 100 μg of KLH (Pierce Chemical Co.) in CFA or intraperitoneally with 115 μg of Pneumovax (Merck). Starting on day 0, mice received 7 daily intraperitoneal injections of 5 mg/kg of either soluble TACI-Fc fusion protein or nonfused Fc as control, and were bled on day 7. Anti-KLH and anti-Pneumovax IgG and IgM were measured in serum by ELISA. In brief, for the measurement of anti-KLH antibodies, plates were coated with KLH in PBS, blocked, and added with test samples or dilutions of standard. Captured anti-KLH IgGs or IgMs were revealed using anti-IgG or anti-IgM biotinylated antibodies and neutravidin-conjugated horseradish peroxidase, and were quantified by comparisons to standards. For the measurement of anti-Pneumovax IgM, plates were coated with Pneumovax using poly-l-lysine, blocked, and added with dilutions of standard and test samples. Captured anti-Pneumovax IgMs were revealed using an anti-IgM biotinylated antibody and neutravidin-conjugated horseradish peroxidase. Results were compared with the Student's *t* test.

Transfection, Immunoprecipitaion, and Electrophoretic Mobility Assays. 293 cell transfection, coimmunoprecipititaion, and Western blot analysis were performed as described (4). For JNK kinase assay, cell lysates were first immunoprecipitated with anti-JNK monoclonal antibody (BD PharMingen). The kinase activity was then determined by using 2 μg of glutathione *S*-transferase (GST)-JUN (Stratagene). Electrophoretic mobility assays were performed as described (18).

Results and Discussion

Using FACS® analysis with an Fc-tagged TALL-1 protein, we found that the human Burkitt's lymphoma RAJI cell line expresses a high level of TALL-1 receptor. A plasmid cDNA expression library was constructed from RAJI mRNA and arrayed in pools of 100 clones. Individual pools were transfected into 293 cells and assayed for the acquisition of Europium-labeled TALL-1 recombinant protein. Out of 3,000 pools, we were able to identify and confirm 6 primary positive pools. The positive binding signals from these six primary pools ranged from a 2–10-fold increase compared with the rest of the pools (data not

shown). Positive pools 13B4 and 13H11 were then subdivided. Both clones encoded full-length TACI, a previously reported TNFR family member identified through its interaction with CAML (17). The single positive clone recovered from pool 13H4 has an extra 7 bp in the 5′ untranslated region of the cDNA insert, compared with the positive clone isolated from pool 13B4. By PCR analysis, the rest of the four primary positive pools also contained full-length TACI DNA sequence, which likely contributed to the TALL-1 binding activities. The interaction of TACI with TALL-1 was further confirmed by FACS® analysis. 293 T cells were transfected with control vector or TACI expression vector. Transfected cells were then stained with Fc-tagged TALL-1 protein followed by FITC-conjugated secondary antibody. TALL-1 bound only to TACI-transfected 293 cells, but not vector-transfected cells (Fig. 1 B).

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  1 MSGLGRSRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGTCMSC 50  hTACI
                                .:|  ||.:||||    .:.||
  1 ..........................MAMAFCPKDQYWDSSRKSCVSC 22  mTACI
               .         .         .         .
 51 KTICNHQSQRTCAAFCRSLSCRKEQGKFYDHLLRDCISCASICGQHPKQC 100 hTACI
      |. .|||||  ||: :.||||||::|||||  |:|| | | |||.||
 23 ALTCSQRSQRTCTDFCKFINCRKEQGRYYDHLLGACVSCDSTCTQHPQQC 72  mTACI
               .         .         .         .
101 AYFCENKLRSPVNLPPELRRQRSGEVENNSDNSGRYQGLEHRGSEASPAL 150 hTACI
    |:||| : ||  || ||| | ..|||  ||||||:|| ||
 73 AHFCEKRPRSQANLQPELGRPQAGEVEVRSDNSGRHQGSEHG........ 114 mTACI
               .         .         .         .
151 PGLKLSADQVALVYSTLGLCLCAVLCCFLVAVACFLKMRGDPCSCQPRSR 200 hTACI
    |||:||.||. | | |||.||||: ||||||.| ||: ||:|   || .
115 PGLRLSSDQLTL.YCTLGVCLCAIFCCFLVALASFLRRRGEPLPSQP.AG 162 mTACI
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201 PRQSPAKSSQDH..AMEAGSPVSTSPEPVETCSFCFPECRAPTQESAVTP 248 hTACI
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163 PRGSQANSPHAHRPVTEACDEVTASPQPVETCSFCFPERSSPTQESA... 209 mTACI
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249 GTPDPTCAGRWGCHTRTTVLQPCPHIPDSGLGIVCVPAQEGGPGA 293      hTACI
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210 ....PRSLGIHG.FAGTAAPQPCMRATVGGLGVLRASTGDARPAT 249      mTACI
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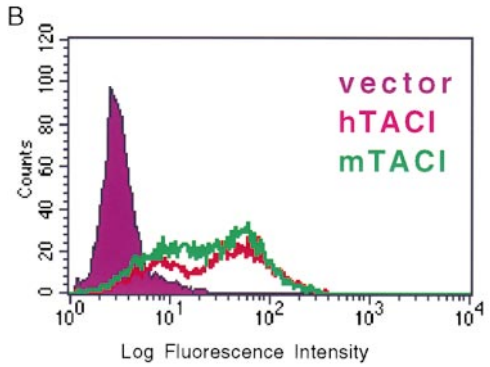

Figure 1. TACI is the cell surface receptor for TALL-1. (A) Comparison of human and murine TACI. The amino acid sequences of human and murine TACI are aligned. Transmembrane regions are underlined. (B) FACS® analysis of TALL-1 binding to TACI-transfected 293 cells. 293 cells (3×10^5) were transiently transfected with vector, human TACI, or murine TACI expression vector. After 24 h, cells were first exposed to 1 μg/ml Fc-tagged TALL-1 protein, then stained with FITC-conjugated goat $F(ab')_2$ anti–human IgG.

A20, a mouse B lymphoma cell line, exhibited high TALL-1 binding activity as determined by FACS® analysis with Fc-tagged TALL-1 (data not shown). The mouse TACI cDNA was subsequently isolated from A20 cDNA library using human TACI cDNA as a probe. The mouse TACI gene encodes a protein of 249 amino acids, lacking 30 amino acids at the NH_2 terminus compared with the human TACI (Fig. 1 A). Human and mouse TACI share 54% identity overall. Six cysteines within the cysteine-rich repeats in the extracellular domain are spatially conserved in both species. Of note, the intracellular domains of human and murine TACI are poorly conserved except for a region of 20 amino acids (Fig. 1 A). The interaction between TALL-1 and mouse TACI was confirmed by 293 cell transfection with mouse TACI and subsequent FACS® analysis with Fc-tagged TALL-1 protein (Fig. 1 B).

Previous studies by von Bulow and Bram (17) showed expression of TACI on B cells and activated T cells. The B cell expression of TACI correlates with TALL-1 B cell binding and stimulatory activities (11, 13, 14). We used PBMCs from healthy donors to determine if TALL-1 also binds activated T cells. As shown in Fig. 2, T cells express

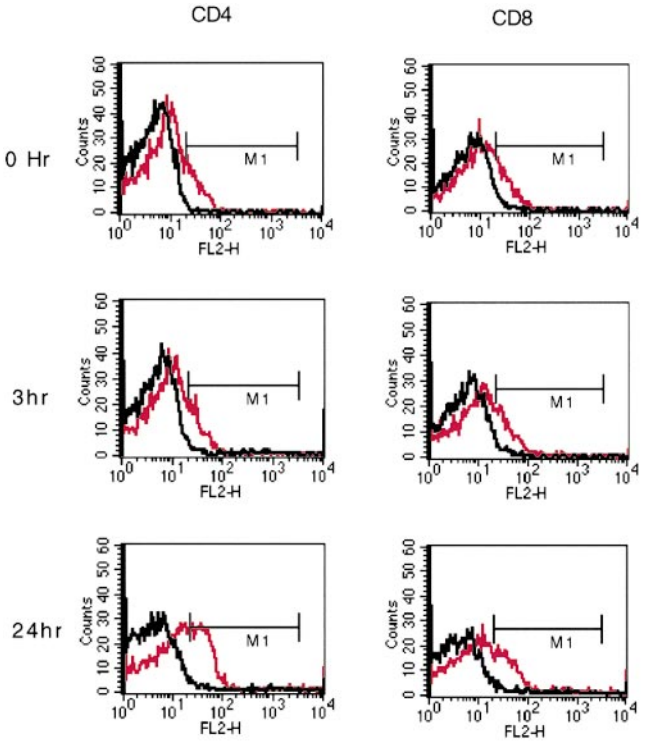

Figure 2. Expression of TALL-1 receptor on activated CD4- and CD8-positive T cells. PBMCs from healthy donors were activated using anti-CD3 antibody (1 μg/ml) for the indicated period of time, and cell surface receptors for TALL-1 were examined by flow cytometry using Flag-tagged TALL-1, anti-Flag biotin antibody, and streptavidin-PE reagents (red histogram). Anti-Flag biotin antibody and streptavidin-PE reagents were used as control for each set of experiments (black histogram). CD4- or CD8-positive T cells were gated to analyze expression of TALL-1 receptor on specific cell types. FL2-H represents log fluorescence intensity.

very low levels of TALL-1 receptors. Upon activation with anti-CD3 antibody, an increase in TALL-1 staining was found in both CD4 and CD8 T cells. The expression of TALL-1 receptors was higher on activated CD4 cells compared with CD8 T cells at all of the time points studied after activation. The biological role of TACI on activated T cells remains to be determined. In conclusion, the TALL-1 binding specificity correlates with TACI expression profile, supporting the fact that TACI is a receptor for TALL-1.

Soluble human TACI recombinant protein (amino acids 1–165) fused with a COOH-terminal His-tag was generated in *E. coli*. Gel filtration analysis indicated that the soluble receptor has a molecular mass of 24 kD, the size of a monomer. The binding kinetics of TALL-1 and TACI were examined by BIAcore analysis. Human and murine TALL-1 bind to human TACI with an affinity of 0.2 nM and 0.3 nM, respectively. Unlike other TNFR family members, both human and murine TACI have an extra-long stalk region of ~60 amino acids following the cysteine repeats at the extracellular domains. This stalk region is not required for the ligand-binding activity. When deleted, the remaining cysteine-rich repeat region (amino acids 1–105) retained TALL-1 binding activity (data not shown).

We recently reported that TALL-1 is a potent B cell co-stimulatory factor with an ED_{50} of ~3 ng/ml. TALL-1–mediated B cell proliferation was completely blocked by soluble TACI extracellular domain protein (Fig. 3). This inhibitory effect was very potent. In the presence of an equal molar ratio of TALL-1 and TACI, B cell proliferation mediated through TALL-1 was inhibited by 50%. This inhibitory effect by soluble TACI protein was not observed when B cell proliferation was induced by anti-CD40 antibody or LPS (Fig. 3). The specific inhibition of TALL-1–mediated B cell proliferation by soluble TACI protein strongly suggests that TACI serves as a physiological cell surface receptor for TALL-1.

We next examined the effect of soluble TACI protein treatment on the production of anti-KLH and anti-Pneumovax antibodies in mice. It is well known that IgG production in response to KLH requires T cell help, whereas anti-Pneumovax IgM production is T cell independent (19). Treatment with soluble TACI protein fused with Fc

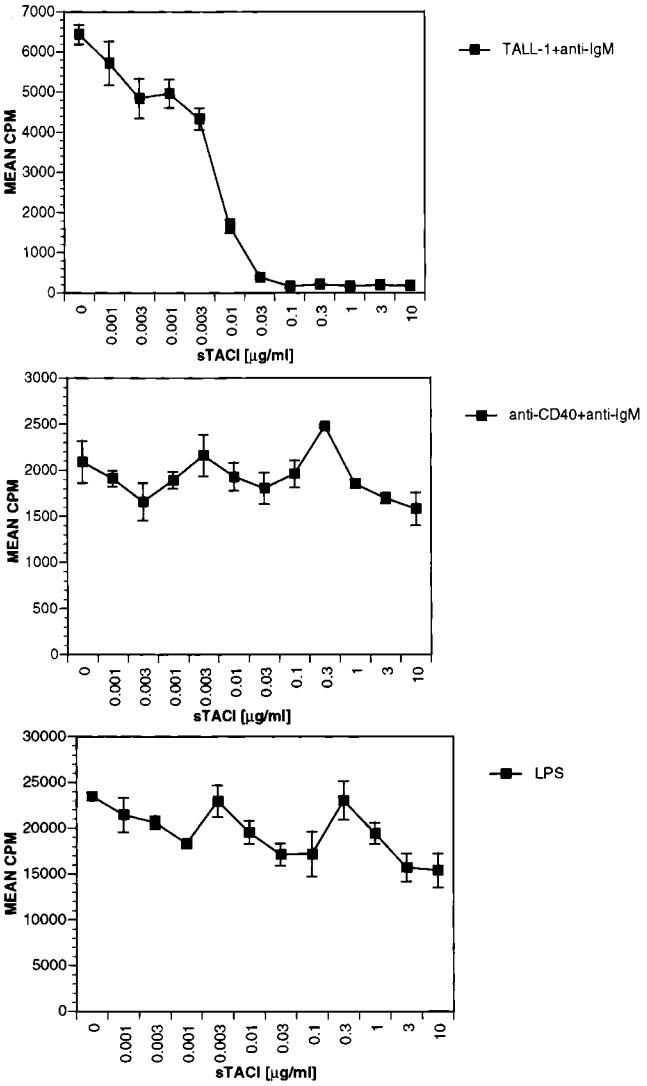

Figure 3. Soluble TACI protein specifically inhibited TALL-1–mediated B cell proliferation. Purified B cells (10^5) from B6 mice were cultured in triplicates in 96-well plates with the indicated amounts of soluble TACI extracellular domain protein in the presence of 10 ng/ml TALL-1 plus 2 μg/ml anti-IgM antibody (top), 1 μg/ml anti-CD40 antibody plus 2 μg/ml anti-IgM antibody (middle), or 0.5 μg/ml LPS (bottom) for a period of 4 d. Proliferation was measured by radioactive [^{3}H]thymidine uptake in the last 18 h of pulse. Data shown represent mean ± SD of triplicate wells.

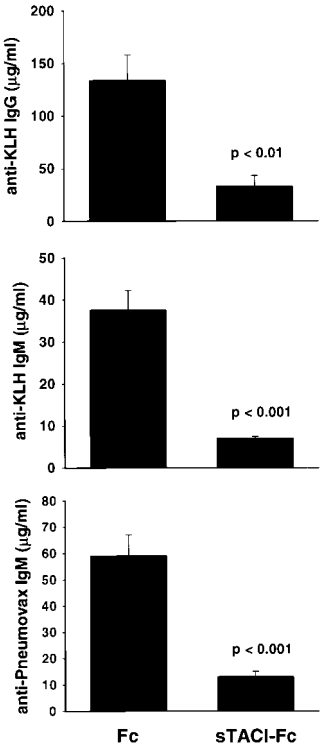

Figure 4. Soluble TACI-Fc fusion protein inhibits anti-KLH and anti-Pneumovax antibody production. Mice (n = 7) were treated with 5 mg/kg TACI-Fc fusion protein or nonfused Fc protein each day for 7 d. Serum levels of anti-KLH IgG and IgM and anti-Pneumovax were measured on day 7 by ELISA.

significantly inhibited the production of anti-KLH and anti-Pneumovax antibodies. Serum levels of anti-KLH IgG and IgM were reduced approximately four- and fivefold, respectively, in the soluble TACI-Fc treated mice compared with the control group (Fig. 4). Serum levels of anti-Pneumovax IgM were also about four times lower in the soluble TACI-Fc treated mice than in controls (Fig. 4). These findings suggest that the TALL-1–TACI interaction is involved in the generation of both T cell–dependent and independent humoral responses.

To identify signaling molecules that TACI uses during B cell stimulation, the intracellular domain of TACI was used as bait in the yeast two-hybrid screening of human B cell library. From 8×10^6 transformants, 48 positive clones were recovered. The majority of the positive clones encoded TRAF2. In addition to TRAF2, TACI intracellular domain also interacted with TRAF5 and TRAF6. The TRAF-binding sites were mapped by deletion mutagenesis in a yeast two-hybrid interaction assay (Fig. 5 A). Both TRAF2- and TRAF5-binding sites colocalized within amino acid residues 231–253 of the human TACI intracellular domain. The TRAF6-binding site occupies an overlapping but broader region from amino acid residues 220–253. It remains to be determined if the TRAF6-binding site is physically separated from the TRAF2- and TRAF5-binding sites within this small region. Interestingly, these TRAF binding sites are the only well-conserved regions between human and murine TACI intracellular domain sequences (Fig. 1 A).

TACI was initially reported as a CAML-binding protein isolated using CAML fused with the GAL4 DNA-binding domain as bait in a yeast two-hybrid screening. In our two-hybrid screening of B cell library with TACI intracellular domain, we did not retrieve CAML among the positive binding clones. However, as reported, we were also able to detect coimmunoprecipitation of TACI with myc-tagged CAML from transfected 293 cells (Fig. 5 B). Corresponding to the yeast interaction, TACI was also coimmunoprecipitated with myc-tagged TRAF2, 5, and 6. Incubation of the same transfected lysates with mouse IgG did not coprecipitate TACI proteins (data not shown). Deletion of 60 amino acids from the TACI COOH terminus abolished its interaction with TRAF2, 5, and 6, consistent with the yeast deletion mapping results (Fig. 5 B). Interestingly, the

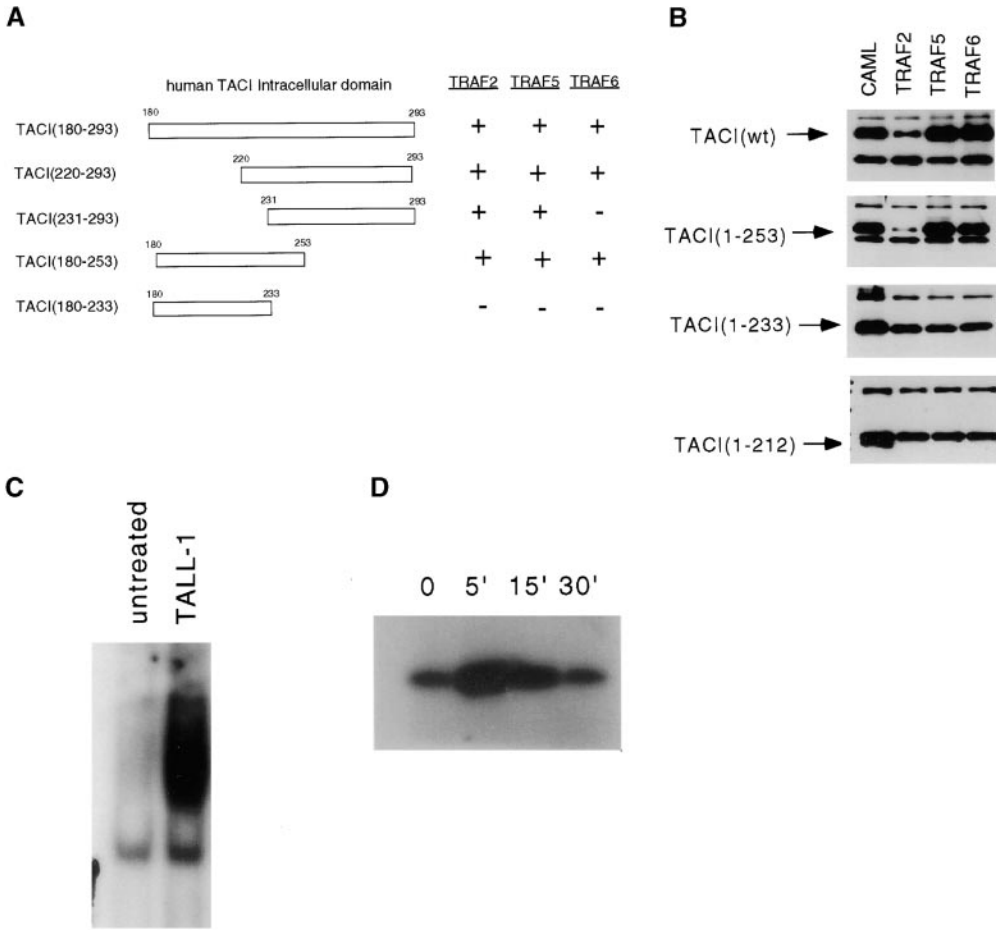

Figure 5. TACI interacts with TRAF proteins and induces NF-κB and JNK activation. (A) Mapping of TACI TRAF-binding domains. Expression vectors encoding full-length or deletion mutants of TACI intracellular domain fused to the GAL4 DNA–binding domain were cotransformed into the HF7C yeast strain with vectors expressing the GAL4 activation fused with TRAF2, 5, and 6. Plus signs represent growth after 1 wk on the selection plates. (B) Coimmunoprecipitation of TACI with TRAF and CAML proteins. 293 cells (3×10^5) were cotransfected with expression vectors directing synthesis of NH_2-terminal Flag-tagged wild-type (wt) TACI or TACI deletion mutants along with myc-tagged CAML, TRAF2, TRAF5, and TRAF6 expression vectors. After 24 h, cell lysates were immunoprecipitated with monoclonal antibody against myc epitope. Coprecipitated Flag-tagged TACI mutants, as indicated by arrows, were detected by immunoblot analysis with anti-Flag monoclonal antibody. For each transfection sample, TACI wild-type or mutants were not detected when mouse IgG was used for the immunoprecipitation (data not shown). (C) NF-κB activation induced by TALL-1. Approximately 10^7 A20 cells were left untreated or were treated with 100 ng/ml TALL-1 for 2 h. Nuclear extracts were prepared, incubated with the ^{32}P-labeled NF-κB oligonucleotide probe, and subjected to electrophoretic mobility shift analysis. (D) JNK activation induced by TALL-1. Approximately 10^6 A20 cells were exposed to 100 ng/ml TALL-1 for the indicated length of time. The cell lysates were immunoprecipitated with monoclonal anti-JNK antibody. Immunoprecipitates were assayed for kinase activity by using GST-JUN as substrate.

same deletion mutant TACI (1–233) still retained CAML-binding activity, suggesting that the TRAF-binding and CAML-binding sites of TACI reside in two separable regions (Fig. 5 B). The TRAF2, 5, and 6 knockout mice will be useful tools to evaluate the biological roles of these TRAF proteins in TALL-1 signaling pathways.

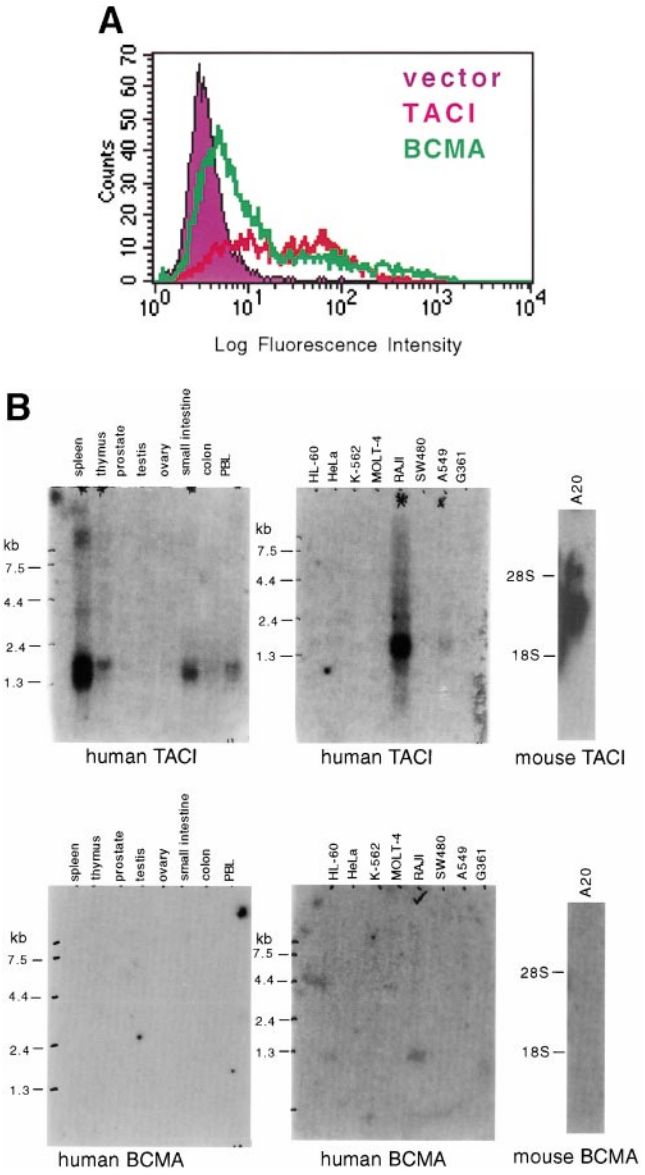

Figure 6. Northern blot analysis of TACI and BCMA. (A) FACS® analysis of TALL-1 binding to TACI- and BCMA-transfected 293 cells. 293 cells (3×10^5) were transiently transfected with vector, human TACI, or human BCMA expression vector. After 24 h, cells were first exposed to 1 μg/ml Fc-tagged TALL-1 protein, then stained with FITC-conjugated goat $F(ab')_2$ anti–human IgG. (B) Northern blot analysis of TACI and BCMA. Full-length coding regions of human and mouse TACI (top) or BCMA (bottom) were generated by PCR and used as probe in the Northern blot analysis of poly A^+ RNA from A20 cells or multiple-tissue Northern blot (CLONTECH Laboratories, Inc.). The blots were exposed to Biomax film (Eastman Kodak Co.) at −80°C for 2 d.

Most TRAF-binding TNFR family members, upon activation by their ligands, induce NF-κB and JNK activation. These two potential signaling events were evaluated in TALL-1–treated A20 cells, which express TACI. NF-κB activation was readily detected from A20 cell nuclear extracts after exposure to TALL-1 for 2 h, as determined by electrophoretic mobility assays with NF-κB oligos (Fig. 5 C). To detect JNK activation, A20 cells were induced with TALL-1 for the indicated periods of time. Activation of JNK was readily detectable after 5 min of TALL-1 treatment, and rapidly decreased after 30 min of exposure (Fig. 5 D). Hence, like many other TRAF-binding TNFR family members, TALL-1 induces NF-κB and JNK activation upon binding to its cell surface receptor TACI, which may then contribute to B cell survival and proliferation.

Our findings clearly demonstrate that TACI is a signaling receptor for TALL-1. This observation was recently reported by Gross et al. (20) during the preparation of this manuscript. In addition to TACI, Gross et al. also demonstrated that B cell maturation antigen (BCMA) is another receptor for TALL-1. We also noted TALL-1 binding to BCMA-transfected 2939 cells (Fig. 6 A). Of note, in TALL-1–responsive A20 cells, BCMA expression was not detectable by Northern blot analysis, whereas TACI expression is high (Fig. 6 B). TACI mRNA was readily detected in RAJI cells, spleen, and other organs rich in lymphoid tissues (Fig. 6 B). In comparison, after the same period of exposure, BCMA expression was weakly detected in RAJI cells, and was not detectable in the other tissues examined (Fig. 6 B). After longer exposure, BCMA mRNA was detected in the small intestine and spleen (data not shown). The observation of the different expression levels of the two receptors may provide some insight into their respective biological roles. However, the generation of specific neutralizing antibodies or knockout mice will provide more useful information in this regard. TALL-1 has been implicated in B cell–mediated autoimmune diseases such as SLE (11,16, and 20). The identification and functional study of TALL-1 receptors provide an advancement in our understanding of B cell survival and proliferation, and represent a clear step forward in the development of potential treatment for these diseases.

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