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Heterozygosity for TACI A144E causes haploinsufficiency and pneumococcal susceptibility in mice

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Abstract

Background—The B cell receptor TACI is important for T-independent antibody responses. One in 200 blood donors are heterozygous for the TACI A181E mutation.

Objective—To investigate the impact on B cell function of TACI A181E heterozygosity in reportedly healthy subjects and of the corresponding TACI A144E mutation in mice.

Methods—Nuclear factor kappa B (NFκB) activation was measured by luciferase assay in 293T cells co-transfected with wild-type (WT) and mutant TACI. TACI driven proliferation, isotype switching and antibody responses were measured in B cells from heterozygous TACI A144E knock-in mice. Mouse mortality was monitored after intranasal pneumococcal challenge.

Results—The levels of natural antibodies to the pneumococcal polysaccharide component phosphocholine (PC) were significantly lower in A181E heterozygous than TACI sufficient Swedish blood donors never immunized with pneumococcal antigens. While overexpressed hTACI A181E and mTACI A144E acted as a dominant negative in transfectants, homozygosity for A144E

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in mice resulted in absent TACI expression in B cells, indicating that the mutant protein is unstable when naturally expressed. A144E heterozygous mice, like TACI$^{+/-}$ mice, expressed half the normal level of TACI on their B cells and exhibited similar defects in APRIL-driven B cell activation, antibody responses to TNP-Ficoll, production of natural antibodies to PC, and survival following intranasal pneumococcal challenge.

Conclusion—These results suggest that TACI A181E heterozygosity results in TACI haploinsufficiency with increased susceptibility to pneumococcal infection. This has important implications for asymptomatic TACI A181E carriers.

Keywords

TACI; CVID; Natural antibodies; pneumococcal susceptibility; B cells

INTRODUCTION

The transmembrane activator and calcium modulator ligand (CAML) interactor (TACI) is a tumor necrosis factor receptor (TNFR) superfamily member expressed on B cells [1]. The TNF family members, B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), are ligands for TACI [2–4]. The extracellular (EC) region of TACI contains two cysteine rich domains (CRDs). Ligand binding causes clustering of the intracellular (IC) domains of TACI, recruitment of signaling molecules that include CAML and TNFR associated factors (TRAF) proteins, and activation of the transcription factors NFAT and NFκB [1, 5–7]. TACI is important in immunoglobulin (Ig) class switching, Ig production, regulation of B cell homeostasis and the antibody response to type II T-independent (TI) antigens, which include natural antibodies to bacterial antigens and the pneumococcal polysaccharide component phosphocholine (PC) [5, 8–10].

TNFRSF13B, which encodes TACI, is mutated in up to 10% of patients with common variable immunodeficiency (CVID) [8, 11, 12]. Most (90%) of these patients are heterozygous for the TACI C104R or A181E mutations. The C104R mutation, located in the second CRD, abolishes ligand binding [8, 11]. The A181E mutation, located in the transmembrane domain, does not affect ligand binding, but severely impairs ligand-induced signaling [13]. B cells from CVID patients with heterozygous C104R and A181E mutations in TACI have impaired response to TACI ligation *in vitro* [8, 11].

About 1 in 200 of reportedly healthy subjects are heterozygous for the A181E mutation (ExAC database, http://exac.broadinstitute.org/variant/17-16843729-G-T). Thus, the A181E mutation is not a cause of CVID but a risk factor and modifier for the disease. The impact of TACI A181E heterozygosity on B cell function in these subjects is not known. We herein show that the levels of natural antibodies to PC in apparently healthy adults subjects never immunized with pneumococcal antigens are significantly lower in A181E heterozygotes compared to TACI sufficient controls. Because humans have complex heterogeneous genetic backgrounds, we investigated the impact of an isolated A144E mutation in mice, which corresponds to the A181E mutation in humans, on the immune response to T-independent (TI) type II antigens in order to determine the mechanistic basis of the impaired anti-PC response in apparently healthy A181E carriers with no CVID. We demonstrate that

heterozygosity for mTACI A144E causes TACI haploinsufficiency and increased susceptibility to pneumococcal infection.

RESULTS

Asymptomatic human carriers of the TACI A181E mutation have impaired natural antibody responses

Antibodies to PC, which is present on all *S. pneumoniae* strains as part of the cell wall polysaccharide [17, 18], are thought to protect against invasive pneumococcal disease in humans [19]. PC antibodies were measured in sera from 14 A181E heterozygotes and 20 TACI sufficient Swedish blood donors who had never been vaccinated with pneumococcal antigens, as pneumococcal vaccination was not performed in Sweden for their age group. Serum levels of natural IgM and IgG antibodies to PC were significantly lower in the A181E heterozygotes compared to controls (Fig 1,A). However, the affinity of anti-PC IgG antibodies was significantly higher in A181E heterozygotes compared with controls (Fig 1,B). Serum levels of natural IgM antibodies to *E. coli* were also significantly lower in the A181E heterozygotes compared to controls (Fig 1,C). Serum levels of IgM, IgG and IgA of antibodies to the T dependent (TD) antigen tetanus toxoid were not significantly different between the two groups (Fig 1,D and data not shown). These results indicate that the heterozygous A181E mutation impairs the natural antibody response to TI bacterial antigens in reportedly healthy human subjects.

Phenotypic analysis of lymphocytes in TACI A144E knock-in mice

TNF family ligands transduce signals though inducing clustering of the IC domain of their receptors, and most TNFRs also have some degree of pre-ligand association, which is thought to facilitate signaling [20, 21]. We examined whether the hTACI A181E and mTACI A144E could assemble with their WT counterparts. Flag-tagged mutants and their Myc-tagged WT counterparts were co-transfected in 293T cells with Flag-tagged and Myc-Tagged WT constructs used as positive controls. The A181E hTACI and A144E mTACI mutants assembled with their WT counterparts, albeit less efficiently than WT TACI with itself (Fig E2,A and B). The assembly of the mutants with WT TACI is not unexpected, given that the extracellular CRD1 domain, which is critical for the pre-association of WT TACI, is intact in these mutants.

We previously showed that the hTACI A181E and mTACI A144E mutations severely impair ligand-induced activation of NFκB in 293T cell transfectants [13]. Additional studies demonstrated that both A181E hTACI and A144E mTACI mutants strongly inhibited the ability of co-transfected WT TACI to activate NFκB in 293 T cells (Fig E2,C and D). Furthermore, TACI driven B cell responses of $TACI^{+/-}$ mice that carry a mTACI A144E expressing transgene were intermediate between those of $TACI^{-/-}$ mice and $TACI^{+/-}$ mice [13]. These results indicate that the mutants, when overexpressed, may act as dominant negatives. However, overexpression systems are not physiologic. To definitively address the effect of the heterozygous A144E mutation on B cell function, we generated gene targeted knock-in mice that carry a *Tnfrsf13b* allele that encodes the TACI A144E mutant in the endogenous locus, mimicking humans heterozygous for the A181E TACI mutation (Fig E1).

Homozygous TACI$^{A144E/A144E}$ mice, heterozygous TACI$^{+/A144E}$ mice and WT TACI$^{+/+}$ littermates, as well as TACI$^{+/-}$ and TACI$^{-/-}$ mice, were bred for more than 10 generations on the C57BL/6 background. None of the mutants differed in growth, weight, or health from their WT littermates, and all had normal lymphocyte cellularity in the thymus, bone marrow (BM), and spleen (data not shown). B cell development in the BM, T and B cell distribution in the spleen, and B cell subsets in the spleen and peritoneal cavity were comparable in all five strains, with the exception, as previously reported [5, 9, 10], of a significant increase in the percentage of B cells with a concomitant decrease in the percentage of T cells in the spleen of TACI$^{-/-}$ mice (Fig E3).

The A144E mutation severely impairs TACI expression in B cells

B cells from TACI$^{A144E/A144E}$ and TACI$^{+/A144E}$ mice expressed comparable levels of *Tnfrsf13b* mRNA as WT B cells, whereas B cells from TACI$^{+/-}$ mice expressed approximately half the WT level of *Tnfrsf13b* mRNA (Fig 2,A). FACS analysis revealed that the intensity of TACI expression on B cells was markedly diminished in TACI$^{A144E/A144E}$ mice and was approximately half normal in TACI$^{+/A144E}$ and TACI$^{+/-}$ mice (Fig 2,B and C). Intracellular FACS analysis revealed approximately half normal TACI expression in B cells from TACI$^{+/A144E}$ and TACI$^{+/-}$ mice, and virtually no TACI expression in B cells from TACI$^{A144E/A144E}$ mice (Fig 2,D and E). These results indicate that the mutant TACI protein is poorly expressed, and demonstrate that the heterozygous A144E mutation results in haploinsufficiency.

TACI$^{+/A144E}$ mice exhibit functional TACI haploinsufficiency *in vitro*

B cell proliferation to APRIL and IgG1 secretion in response to APRIL+IL-4 were significantly and comparably reduced in TACI$^{+/A144E}$ and TACI$^{+/-}$ mice relative to WT controls (Fig 3,A and B). B cells from TACI$^{A144E/A144E}$ and TACI$^{-/-}$ mice failed to respond to APRIL stimulation. There was no evidence of increased apoptosis or cell death in APRIL +IL4 stimulated cultures of B cells from TACI mutant mice compared to WT controls, as determined by Annexin V and propidium iodide staining (data not shown). B cells from all five strains of mice proliferated and secreted IgG1 comparably in response to anti-CD40+IL-4 (Fig 3,A and B). These results indicate that the heterozygous TACI A144E mutation impairs TACI function in B cells *in vitro*.

TACI$^{+/A144E}$ mice have impaired antibody response to TNP-Ficoll, but normal antibody response to TD antigen

TACI$^{-/-}$ mice have decreased serum immunoglobulin levels[9, 13, 22]. Serum IgM, IgG and IgA levels were comparable in TACI$^{+/A144E}$ mice, TACI$^{+/-}$ mice and TACI$^{+/+}$ controls (Fig 4,A). Serum IgM, IgG and IgA levels were significantly lower in TACI$^{A144E/A144E}$ mice compared to TACI$^{+/+}$ controls and not significantly different than those of TACI$^{-/-}$ mice.

TACI is essential for the antibody response to the type II TI antigen TNP-Ficoll in mice, as TACI$^{-/-}$ mice fail to mount IgM and IgG anti-TNP responses to immunization with TNP-Ficoll [9] (Fig 4,B). TACI$^{+/A144E}$ mice, like TACI$^{+/-}$ mice, had significantly and comparably lower IgM and IgG anti-TNP responses to TNP-Ficoll than TACI$^{+/+}$ controls (Fig 4,B). The antibody responses to TNP-Ficoll of TACI$^{A144E/A144E}$ mice were significantly lower than

those of TACI$^{+/A144E}$ and TACI$^{+/-}$ mice, and although they were higher than those of TACI$^{-/-}$ mice, the difference was not significant. These results indicate that the heterozygous TACI A144E mutation impairs the B cell response to type II TI antigen *in vivo*.

The anti-TNP antibody response to TNP-KLH in TACI$^{+/A144E}$ mice, TACI$^{A144E/A144E}$ TACI$^{+/-}$ mice and TACI$^{-/-}$ mice was comparable to that of WT controls when measured on day 28 post-immunization (data not shown), consistent with the previously reported normal response of TACI$^{-/-}$ mice to TNP-KLH[22]. It was recently reported that TACI$^{-/-}$ mice fail to sustain the anti-NP IgG antibody response to the TD antigen NP-OVA [14]. We observed no significant decrease in the levels of IgG anti-NP antibodies on days 35 and 56 post-immunization with OVA-NP in any of the mutant strains compared to WT controls (Fig 4,C). The normal response of TACI$^{+/A144E}$ mice to TD antigens indicates that the mutant does not exert non-specific effects on B cell differentiation into antibody producing cells.

TACI$^{+/A144E}$ mice have decreased levels of natural antibodies to PC and increased susceptibility to pneumococcal inhalation challenge

Antibodies to PC protect unimmunized mice from death following pneumococcal challenge [23]. Serum levels of natural IgM and IgG antibodies to PC were readily detected in TACI$^{+/+}$ mice but were virtually absent in TACI$^{-/-}$ mice, indicating that this response is largely TACI dependent (Fig 5,A). Serum levels of natural IgM and IgG antibodies to PC were significantly lower in TACI$^{+/A144E}$ mice, and TACI$^{+/-}$ mice compared to TACI$^{+/+}$ controls (Fig 5,A). These results demonstrate that the heterozygous A144E mutation impairs the natural antibody response to PC.

We next examined the impact of the heterozygous A144E mutation on the response of unimmunized mice to pneumococcus. Analysis of Kaplan-Meier survival curves following intranasal challenge of unimmunized, pneumococcus-naive mice with *S. pneumoniae* serotype 3 revealed that 78% (18 of 23) of TACI$^{+/+}$ mice survived 10 days post-challenge (Fig 5,B). In contrast none of 12 TACI$^{-/-}$ mice survived the challenge, indicating that protection of naïve mice against death from intranasal pneumococcal challenge is largely TACI dependent. The survival rates of TACI$^{+/A144E}$ heterozygous mice (33%, 4 of 12 mice) and TACI$^{+/-}$ mice (38%, 5 of 13 mice) on day 10 post-challenge were significantly lower than that of TACI$^{+/+}$ controls, but did not significantly differ from each other (Fig 5,B). These results demonstrate that the heterozygous A144E mutation increases the susceptibility of naïve mice to death following intranasal pneumococcal challenge.

DISCUSSION

We show that the levels of natural antibodies to PC, which are implicated in resistance to pneumococcal infection, are significantly lower in reportedly healthy TACI A181E heterozygous adults and that mice heterozygous for TACI A144E, the murine counterpart to A181E, demonstrate increased susceptibility to pneumococcal infection. These results suggest that heterozygosity for the TACI A181E mutation may confer an increased risk for pneumococcal infections.

We identified reportedly healthy adult Swedish blood donors who were heterozygous for the TACI A181E mutation, consistent with the observation that relatives of CVID patients who also carry the A181E mutation can be clinically healthy [24]. Blood donors heterozygous for the TACI A181E mutation had significantly lower levels of natural IgM and IgG antibodies to PC compared to TACI sufficient controls. However, the affinity of IgG antibodies to PC was significantly increased in the A181E carriers compared to controls. It was previously reported that the affinity of IgG antibodies to T-dependent antigens is higher in $TACI^{-/-}$ mice compared to WT controls [14]. Ligation of high affinity B cell receptors (BCR) by antigen delivers an apoptotic signal [25]. TACI has also been reported to deliver an apoptotic signal to B cells while BAFF-R which shares common ligands with TACI delivers a survival signal [5, 26–28]. Decreased TACI signaling and/or increased BAFF-R signaling may allow better survival of B cells that express high affinity BCRs resulting in the production of higher affinity antibodies. It would be of interest to study the post-vaccination antibody response to pneumococcal polysaccharides in these apparently healthy carriers of the A181E mutation. However, this was not possible, since all subjects were de-identified. TACI A181E carriers also had significantly lower levels of natural IgM antibodies to *E. coli* antigens, raising the possibility that they may be susceptible to infections with organisms other than pneumococcus. The ~0.7% incidence of A181E heterozygosity in the cohort of Swedish blood donors we studied is more than 100 fold higher than the 1 in 25,000 estimated incidence of CVID in Sweden. Thus, the majority of the A181E heterozygotes studied are unlikely to be closely related to patients with CVID, suggesting that their impaired natural antibody response is not caused by genes or factors associated with CVID.

Our studies with TACI A144E knock-in mice clearly indicate that the TACI A144E mutant protein is poorly expressed in B cells. This is consistent with the location of this mutation at a predicted stability center of the protein [29]. Poor expression of the A144E mutant protein explains the observation that the level of TACI surface and intracellular protein in B cells of $TACI^{+/A144E}$ heterozygous mice was about half that of WT B cells, comparable to that of B cells from $TACI^{+/-}$ mice. Thus, the heterozygous A144E mutation results in haploinsufficiency of surface TACI expression in mouse B cells. TACI surface expression is reduced in B cells from CVID patients and their asymptomatic relatives heterozygous for the A181E TACI mutation compared to normal controls [24].

Mice heterozygous for the A144E TACI mutation displayed functional TACI haploinsufficiency. B cell proliferation in response to APRIL stimulation, IgG1 secretion in response to stimulation with APRIL+IL-4 and, more importantly, antibody responses to the type II TI antigen TNP-Ficoll, but not to the TD antigens NP-OVA and TNP-KLH were significantly reduced in heterozygous $TACI^{+/A144E}$ mice and similar to those of haploinsufficient $TACI^{+/-}$ mice. Consistent with their poor expression of TACI, $TACI^{A144E/A144E}$ homozygous mice, like $TACI^{-/-}$ mice, had markedly diminished TACI expression on B cells and a virtually absent response to APRIL *in vitro*. Unlike $TACI^{-/-}$ mice, $TACI^{A144E/A144E}$ mice had residual anti-TNP-Ficoll antibody responses and no expansion of splenic B cells, possibly, suggesting the residual presence of some functionally relevant receptors in these mice

We showed that TACI is critical for the production of natural IgM and IgG antibodies to PC in mice, as evidenced by the virtual absence of these antibodies in the serum of $TACI^{-/-}$ mice. While we did not examine the opsonic activity of sera from the mice for pneumococcal bacteria *in vitro*, we demonstrated that TACI is critical for the survival of unimmunized mice following pneumococcal intranasal challenge, as all $TACI^{-/-}$ mice succumbed after challenge compared to 22% of WT controls. Both the levels of IgM and IgG antibodies to PC, and the fraction of mice that survived pneumococcal challenge, were significantly and similarly reduced in $TACI^{+/A144E}$ mice and $TACI^{+/-}$ mice compared to WT controls. Thus TACI haploinsufficiency caused by the heterozygous A144E TACI mutation, or loss of a TACI allele, significantly increases the susceptibility of mice to pneumococcal disease.

According to the Center for Disease Control, pneumococcal disease is an important health problem accounting for 400,000 hospitalizations per year in U.S. and with a case fatality rate of 5–7% in the elderly (http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/pneumo.pdf). Our findings in $TACI^{+/-}$ mice suggest that lack of expression of TACI encoded by a mutant *TNFRSF13B* allele in humans may impair their antibody response to pneumococcus, and increase susceptibility to pneumococcal infection. Consistent with this hypothesis, patients with the Smith-Magenis syndrome, who have a chromosome 17p11.2 microdeletion that includes *TNFRSF13B,* have decreased TACI surface expression, a poor response to pneumococcus vaccination and recurrent respiratory tract infections [30]. While one needs to draw a cautious conclusion regarding human susceptibility to a given microorganism based on murine data, the A181E carriers, like the A144E knock-in mice, had significantly lower levels of natural antibodies to PC. Antibodies to PC, which is present on all *S. pneumoniae* strains as part of the cell wall polysaccharide, are thought to protect against invasive pneumococcal disease in humans [19]. Thus, it is not far-fetched to propose that the increased susceptibility of the A144E mice to *S. pneumoniae* challenge is relevant to A181E carriers. It would be of interest to study over time whether healthy adults who carry the A181E mutation, including those related to CVID patients with this mutation, are at increased susceptibility to pneumococcal infections and to examine healthy individuals who have had a pneumococcal infection for A181E heterozygosity

Our results suggest that TACI A181E carriers, like $TACI^{+/A144E}$ mice, may be more susceptible to infection with pneumococcus and possibly other polysaccharide encapsulated bacteria. Since TACI function is also disrupted in mice heterozygous for TACI C76R, the counterpart of the human TACI C104R mutation [31], this mutation, like the A181E mutation, may also increase the risk of infection. This would have important implications for the ~1% of individuals who carry one of these TACI mutations.

METHODS

TACI expression and co-immunoprecipitation in transfectants

293T cells were transfected with Myc-tagged WT hTACI or mTACI, and either Flag-tagged WT hTACI, A181E hTACI, WT mTACI or A144E mTACI, using FuGENE 6 (Roche). Forty-eight hours later, lysates were examined for expression and co-immunoprecipitation of Myc-tagged and Flag-tagged proteins as previously described [7].

NFκB reporter assay

293T cells were transfected with 100 ng WT or mutant TACI expression plasmid alone or in combination, along with 100 ng NFκB-luciferase reporter plasmid and 10 ng control pRL-TK plasmid (Promega). Four hours later, trimeric ZZ-APRIL (50 ng/mL a gift from ZymoGenetics) was added. Reporter gene activity was determined 20 hours later using a dual-luciferase reporter assay system (Promega) as previously reported [22].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

APRIL	a proliferation-inducing ligand
BM	bone marrow
CAML	calcium modulator ligand
CFP	cyan fluorescent protein
CRD	cysteine rich domain
CVID	Common variable immunodeficiency
EC	Extracellular
FRET	Fluorescence resonance energy transfer
IC	Intracellular
NFκB	Nuclear factor kappaB
NFAT	nuclear factor of activated T cells
NP-OVA	4-hydroxy-3-nitrophenyl acetyl-ovalbumin
PC	Phosphocholine
TACI	Transmembrane activator and calcium modulator ligand interactor
TI	T independent
TD	T dependent
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor

TNP-KLH	trinitrophenyl-keyhole limpet hemocyanin
WT	wild type
YFP	yellow fluorescent protein

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Key messages

- #x02022; TACI A181E heterozygosity in asymptomatic subjects is associated with decreased levels of natural antibodies to the pneumococcal polysaccharide phosphocholine and *E. coli* antigens.
- Heterozygosity for mTACI A144E, which corresponds to hTACI A181E, results in TACI haploinsufficiency with impaired production of natural antibodies to phosphocholine, and increased susceptibility to pneumococcal infection.

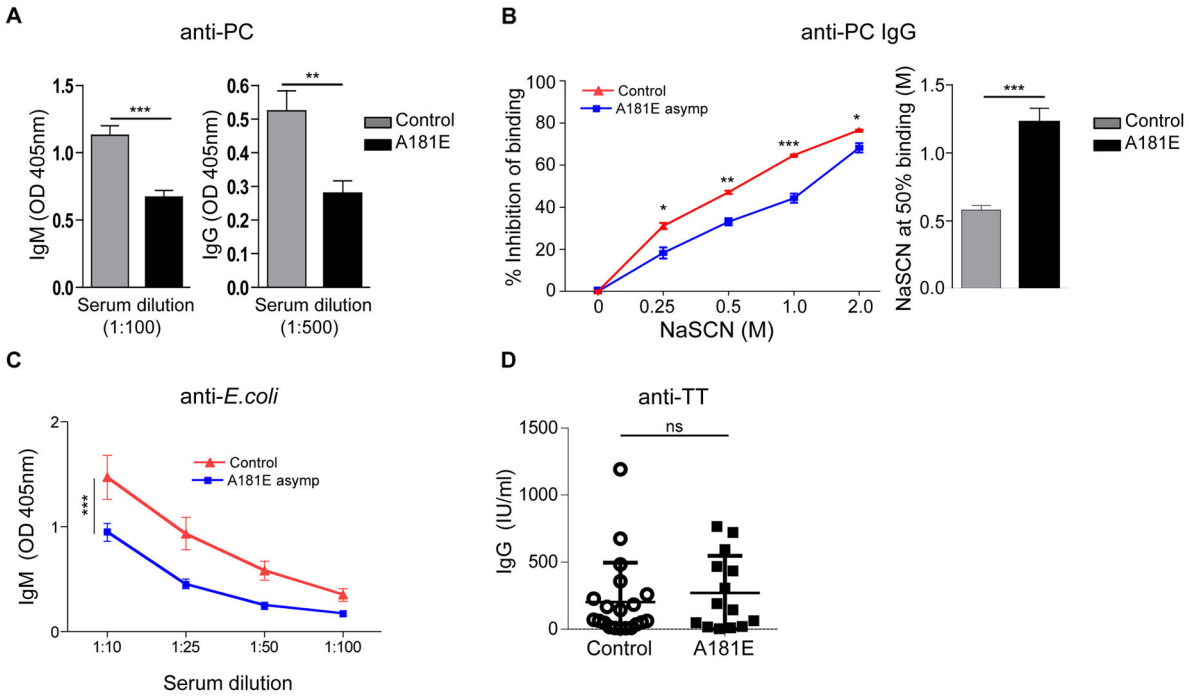

Figure 1. Blood donor carriers of the TACI A181E mutation have impaired natural antibody responses

A, Phosphocholine (PC) specific IgM and IgG antibody, **B.** Inhibition of anti-PC IgG binding to PC by increasing concentrations of NaSCN (left panel) and NaSCN molarity resulting in 50% inhibition of the O.D. (right panel). **C,** *E. coli* specific IgM and **D,** TT specific IgG in sera from Swedish asymptomatic subjects carrying a heterozygous TACI A181E mutation (n=14) and healthy controls (n=20). Data are expressed as OD at 405 nm or IU/ml. Sera were used at 1:100 dilution in A (for IgM), B, and D, and 1:500 dilution in A (IgG). Columns or symbols and bars in A–D represent means ± SEM. * $p<0.5$, ** $p<0.01$, *** $p<0.001$ by Student's *t*-test.

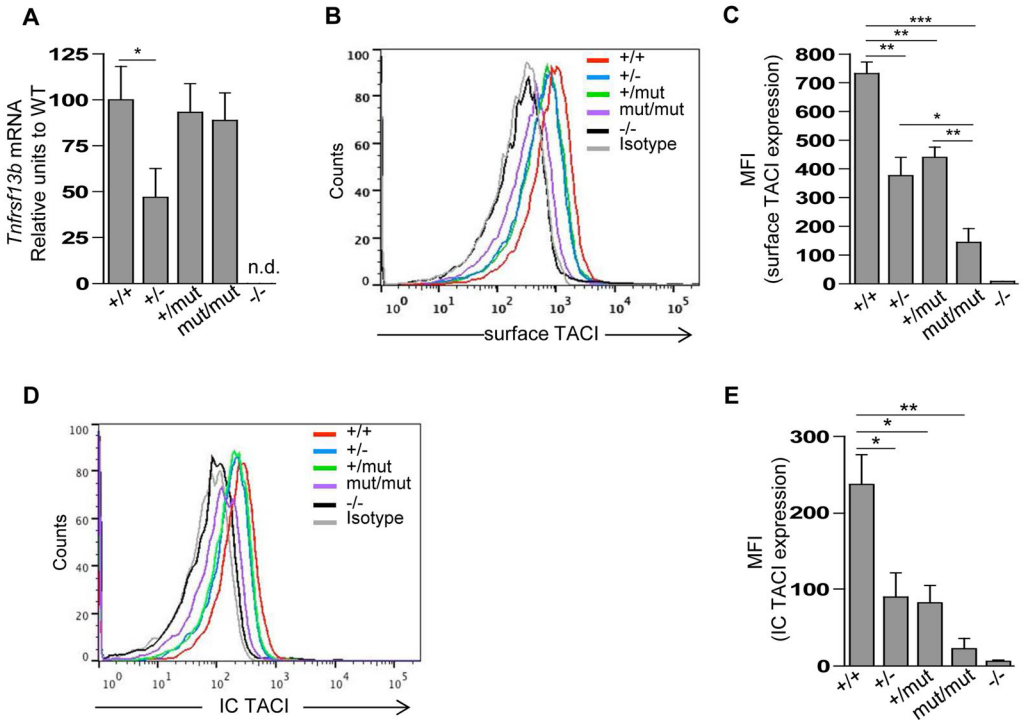

Figure 2. The A144E mutation impairs TACI surface expression, but not mRNA expression, in mouse B cells

A, qRT-PCR analysis of *Tnfrsf13b* mRNA levels in purified $B220^+$ splenic B cells from $TACI^{+/+}$ (+/+), $TACI^{+/-}$ (+/−), $TACI^{+/A144E}$ (+/mut), $TACI^{A144E/A144E}$ (mut/mut), and $TACI^{-/-}$ (−/−) mice. The mRNA expression of *Tnfrsf13b* compared to that of the housekeeping gene *Gapdh* is shown as a percentage of the ratio in B cells from $TACI^{+/+}$ WT controls. **B and C,** Representative FACS analysis (B) and pooled data (C) of the mean fluorescence intensity (MFI) of TACI surface expression on gated $B220^+$ splenic B cells. **D and E,** Representative FACS analysis (D) and pooled data (E) of intracellular TACI expression in gated $B220^+$ peripheral B cells. MFI data presented are the net value of TACI expression (MFI of TACI minus MFI of isotype control). Columns and bars in A, C and E represent mean ± SEM (n=3–5 mice per group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by Student's *t*-test n.d.= not detected.

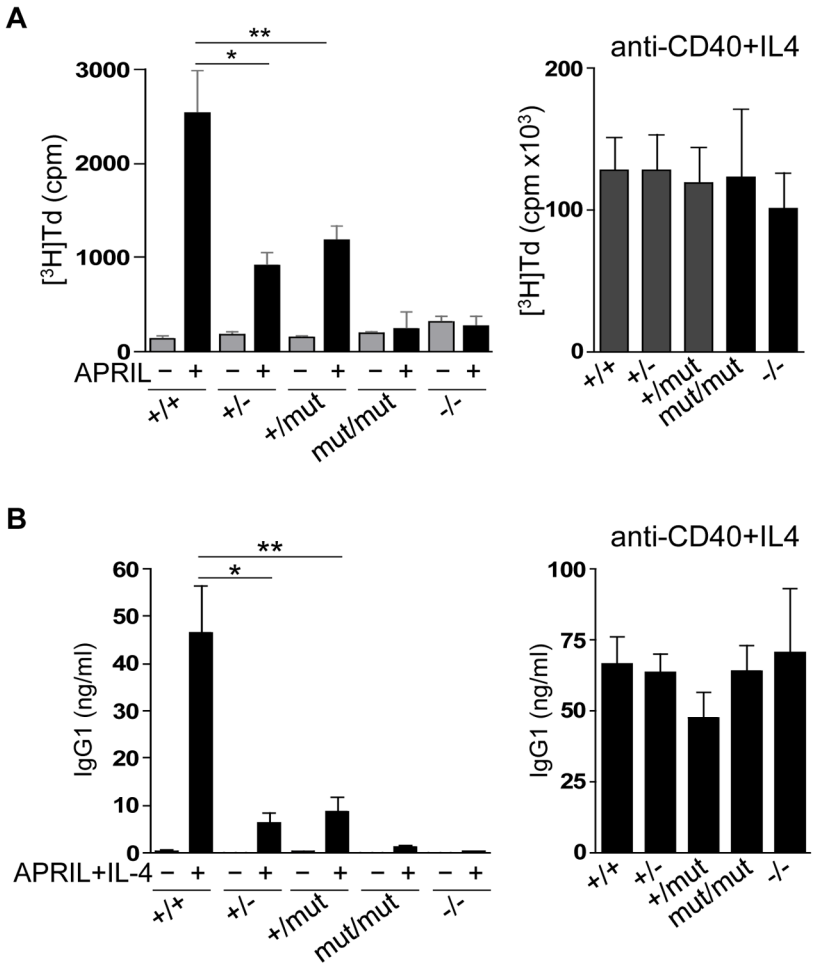

Figure 3. Proliferation and *in vitro* immunoglobulin production in response to APRIL are impaired in B cells from A144E TACI mutant mice

A and B, Proliferation in response to APRIL (A) and IgG1 secretion in response to APRIL +IL-4 (B) by splenic B cells from $TACI^{+/+}$ (+/+) $TACI^{+/-}$ (+/−), $TACI^{+/A144E}$ (+/mut), $TACI^{A144E/A144E}$ (mut/mut), and $TACI^{-/-}$ (−/−) mice. Stimulation with anti-CD40+IL-4 was used as a control. Columns and bars represent means ± SEM (n= 5–7 mice per group). * $p<0.05$, ** $p<0.01$ by Student's *t*-test.

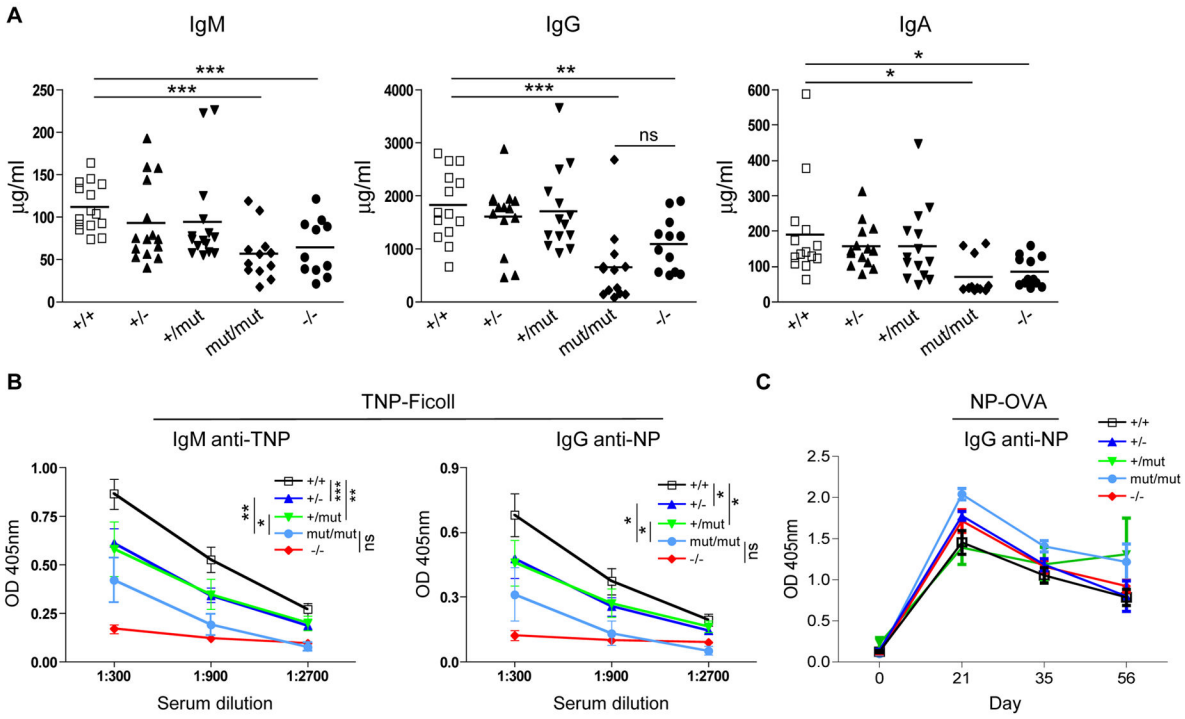

Figure 4. Serum immunoglobulin levels and antibody responses to TNP-Ficoll in A144E TACI mutant mice

A, Serum IgM, IgG and IgA levels from non-immunized 8–12 week-old $TACI^{+/+}$ (+/+), $TACI^{+/-}$ (+/−), $TACI^{+/A144E}$ (+/mut), $TACI^{A144E/A144E}$ (mut/mut) and $TACI^{-/-}$ (−/−) mice. Each symbol represents an individual mouse and the horizontal lines indicate the mean (n=11–15 mice per group). **B,** IgM and IgG anti-TNP responses 14 days after immunization with TNP-Ficoll. **C.** IgG anti-NP response after immunization with NP-OVA. Data in B and C are presented as the optical density (OD) readings at 405 nm. Symbols and bars represent mean ± SEM (n= 5–11 mice per group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns= not significant. Student's *t*-test was used in A, and two-way ANOVA was used in B.

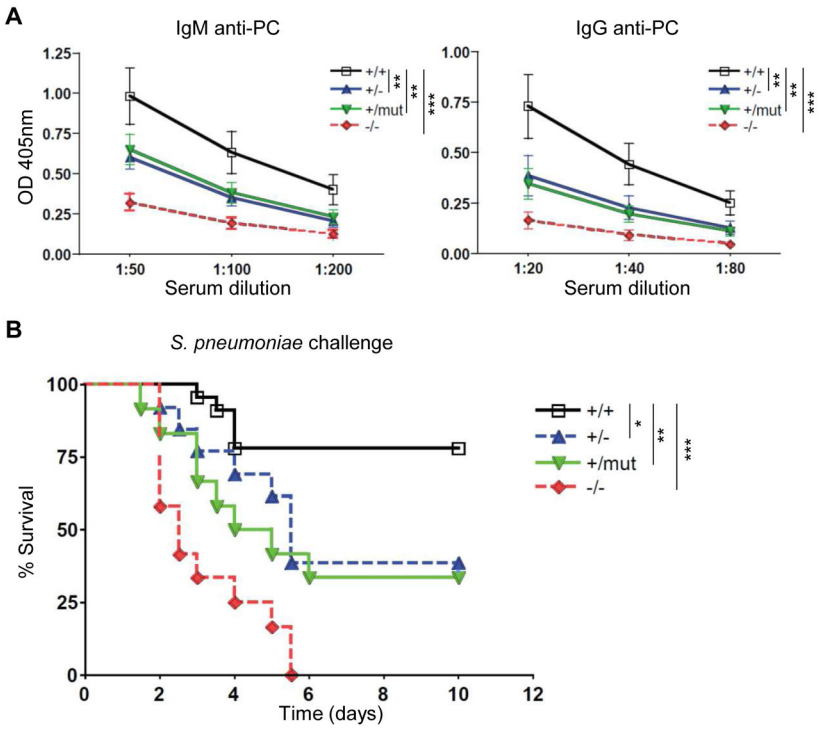

Figure 5. Decreased levels of natural antibodies to phosphocholine and increased susceptibility to pneumococcal inhalation challenge in A144E TACI mutant mice

A, IgM (left) and IgG (right) PC specific antibody levels in sera from non-immunized 8–12 week-old $TACI^{+/+}$ (+/+) $TACI^{+/-}$ (+/−), $TACI^{+/A144E}$ (+/mut), and $TACI^{-/-}$ (−/−) mice expressed as OD at 405 nm. n=7 each group. **B,** Kaplan-Meier survival curves following intranasal challenge of 8–12 week-old unimmunized naïve $TACI^{+/+}$ (+/+), $TACI^{+/-}$ (+/−), $TACI^{+/A144E}$ (+/mut) and $TACI^{-/-}$ (−/−) mice with a *S. pneumoniae* serotype 3 strain (n= 12–23 per group). Symbols and bars in A indicate mean ± SEM and symbols in B represent the percent of surviving mice. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Two-way ANOVA was used in A and Log-rank (Mantel-Cox) test was used in B.