Published in final edited form as:
J Allergy Clin Immunol. 2007 October ; 120(4): 885–891.

Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) enhances CD40 driven plasma cell differentiation

Emanuela Castigli, Ph.D, **Stephen A. Wilson, B.Sc.**, **Abdallah Elkhal, Ph.D**, **Esra Ozcan, M.D.**, **Lilit Garibyan, B.Sc.**, and **Raif S. Geha, M.D.***
Division of Immunology, Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA

Abstract

Background—Transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI) is a receptor used by B-cell activating factor of the TNF family (BAFF) and a proliferation inducing ligand (APRIL) to induce isotype switching independently of CD40 and is mutated in patients with common variable immunodeficiency (CVID).

Objective—To determine whether TACI and CD40 cooperate in inducing class switch recombination (CSR) and immunoglobulin production.

Methods—Naïve mouse B cells were stimulated with suboptimal concentrations of anti-CD40 +IL-4 in the presence or absence of APRIL or anti-TACI. IgG1 and IgE production was measured by ELISA. mRNA for $C\gamma_1$ and Cε germ line transcripts, activation-induced cytidine deaminase (*AICDA*) and mature γ_1 and ε transcripts were measured by RT-PCR. Plasmablasts were enumerated by syndecan-1/CD138 staining. Interferon regulatory factor 4 (IRF4), B lymphocyte-induced maturation protein 1 (Blimp1) and IL-6 mRNA expression was measured by quantitative PCR.

Results—TACI ligation enhanced IgG_1 and IgE secretion by naïve murine B cells stimulated by anti-CD40+IL-4, with little effect on B cell proliferation or CSR. In contrast, TACI ligation of anti-CD40+IL-4 stimulated B cells induced a significant increase in syndecan-1/CD138 positive cells. TACI ligation caused a modest, but significant increase in the expression of IRF4, with no detectable change in Blimp1 expression.

Conclusion—TACI and CD40 signaling converge to promote B cell differentiation into plasmabalsts.

Clinical implications—Our data suggest that TACI dysfunction could contribute to the impaired antibody response to T dependent antigens in CVID.

CAPSULE SUMMARY—This work shows that CD40 and TACI cooperate to promote B cell differentiation into plasma cells and increase immunoglobulin production.

*Corresponding author: Raif S. Geha, Division of Immunology, Children's Hospital, One Blackfan Circle, Boston, Massachusetts 02115, USA. Tel. (617) 919 2482; Fax: (617) 730 0528; E-mail: Raif.Geha@childrens.harvard.edu.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript

Keywords

CD40; TACI; CVID; plasma cell; Immunoglobulin; B cells

INTRODUCTION

Humoral immune response to antigens is characterized by the production of high affinity antibodies by B lymphocytes, which terminally differentiate into plasma cells. B cells encounter antigens in the secondary lymphoid organs where they proliferate and undergo maturation and differentiation that includes CSR, affinity maturation and differentiation into memory B cells or antibody-secreting plasma cells. These processes are initiated and regulated by signals derived from specific ligand-receptor interactions such as between CD40 ligand (CD40L, CD154) and its receptor CD40, between APRIL (CD256, TNFSF13) and BAFF (CD257, TNFSF13B) and their receptor TACI (CD267, TNFRSF13B)[1, 2] and between Toll-like receptor (TLR) ligands and their receptors expressed on B cells[3].

Differentiation of activated B cells into plasma cells involves repression of genes typically expressed by germinal center (GC) B-cells, such as *Pax5, AICDA* and *Bcl-6*, and upregulation of plasma cell specific genes that include the transcription factors *Prdm1*/Blimp1, *IRF4* and X-box binding protein 1 (*XBP-1*), and the proteoglycan syndecan-1/CD138. Both Blimp1 and IRF4 regulate the expression of XBP-1[4], and B cells deficient in Blimp1 or IRF4 are impaired in their ability to undergo plasma cell differentiation[5, 6]. It has been previously shown that Blimp1 expression is strongly induced by TLR4 ligation, but weakly by CD40 ligation in murine B cells[7]. CD40 ligation induces expression of IRF4 in human B cell lines[8], but no data have been published on primary mouse B cells.

We and others[9, 10] recently found that the *TNFRSF13B* gene, which encodes for TACI, is mutated in ~10% of patients with CVID [11], a disorder characterized by recurrent infections, hypogammaglobulinemia, defective antibody production and failure of B cells to differentiate into memory cells and plasma cells[12]. Naïve B cells from CVID patients with TACI mutations fail to secrete IgG and IgA in response to APRIL *in vitro*, but secrete normal amounts of IgG when stimulated by anti-CD40+IL-4[9]. Similar results have been observed in B cells from $TACI^{-/-}$ mice[1]. These results suggest that CD40-induced Ig secretion *in vitro* is independent of TACI. Conversely, TACI induction of Ig secretion *in vitro* is independent of CD40 because it occurs normally in CD40 deficient B cells[1]. Taken together, these data suggest that the CD40 and TACI pathways may activate CSR and Ig secretion in B cells independently of each other. Nevertheless, despite intact CD40 signaling, patients with CVID are impaired in their ability to respond to T dependent antigens and to generate plasma cells[12], raising the possibility that the CD40 and TACI pathways may intersect. In this study we demonstrate that these two pathways cooperate to promote B cell differentiation into plasma cells and increase immunoglobulin production.

MATERIALS AND METHODS

Mice

Balb/c mice were purchased from the Jackson Laboratory. B cell maturation antigen $(BCMA)^{-/-}$ mice and genetically matched (C57/Bl6-129Sv) wild type (WT) controls were previously described[13]. All mice were kept in a specific pathogen free animal facility. All procedures performed on the animals were in accordance with the Animal Care and Use Committee of the Children's Hospital, Boston.

In vitro immunoglobulin production

Naïve B cells from Balb/c, WT and BCMA$^{-/-}$ mice were negatively sorted using a cocktail of biotin-conjugated mAbs to CD43, CD11b, Thy1.2, CD138, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA and IgE and streptavidin magnetic beads (Dynal). B cells were cultured at 1×10^6/ml in RPMI containing 10% FCS, L-glutamine and 50 mM 2-ME (2-Mercaptoethanol) (complete medium). For Ig synthesis, B cells were cultured in one or more of the following: complete medium alone, IL-4 at 50 ng/ml (R&D Systems), suboptimal concentration of anti-CD40 mAb (75 ng/ml, BD-Pharmingen), APRIL at 1 μg/ml, plate bound polyclonal goat anti-TACI at 5 μg/ml or goat IgG at 5 μg/ml (all R&D Systems), and LPS at 10 μg/ml (Sigma). Cells were treated with 5 μg/ml polymyxin B (Sigma) except when LPS was added. After 6 days, supernatants were assayed for IgG_1 and IgE by ELISA[14].

RT-PCR for germ line transcripts (GLT), *AICDA*, and post switch (Iμ-C_H) transcripts

RNA was extracted from 3-day cultured naïve B cells using TRIzol and was reverse transcribed by Supercript II RT (all Invitrogen). PCR primers used, Iμ , $I\gamma_1$, $C\gamma_1$, Iε, Cε, *AICDA* and β_2-microglobulin (β_2m), were previously described[14, 15]. All PCR reactions were performed on 1:1, 1:3 and 1:9 cDNA dilutions for semi-quantitative evaluation. Amplified products were separated on agarose gel and stained with ethidium bromide.

Staining and CFSE (carboxyfluoroscein succinimidyl ester) assay

Naïve B cells from Balb/c mice, stimulated with anti-CD40 for 72 hours, were stained with anti-TACI-PE (phycoeritrin, R&D) or anti-BCMA-FITC (fluorescein Isothiocynate, R&D), or were stimulated for six days as indicated and then stained with anti-CD138-PE and/or anti-IgG_1 PE or biotin-conjugated (BD-Pharmingen) followed by streptavidin-APC (allophycocyanine, eBioscience). For CFSE assays, naïve B cells were loaded with 10 mM CFSE (Molecular Probes), then stimulated and stained as above. For survival assays B cells were stimulated as indicated and stained every day for six days with AnnexinV-FITC (BioVision). All staining were analyzed by FACS.

Quantitative PCR

cDNA obtained as described above was used for real time PCR reactions and run on an ABI Prism 7300 (Applied Biosystems). Taqman primers with 6-carboxyfluorescein-labeled probe for Blimp1, IRF4, IL-6 and the control β_2m were obtained from Applied Biosystems. Relative gene expression among the different samples was calculated as described by Pfaffl[16].

Statistics

p values were determined by the paired t test method using PRISM software.

RESULTS

APRIL increases immunoglobulin production induced by anti-CD40 and IL-4 by engaging TACI

To test the hypothesis that CD40-induced Ig production is increased by engagement of TACI, we first examined the effect of TACI ligation on Ig synthesis induced by a suboptimal concentration of anti-CD40 mAb in negatively sorted naïve splenic B cells from Balb/c mice. To determine the suboptimal concentration of anti-CD40 for our experiments, B cells were stimulated *in vitro* with concentrations of anti-CD40 ranging from 500 to 10 ng/ml and IL-4. Figure 1A shows that 100 ng/ml anti-CD40 consistently achieved optimal induction of IgG_1 and IgE synthesis, while a concentration of 75 ng/ml of anti-CD40 resulted in just suboptimal synthesis of IgG_1 and IgE. Subsequently, we used this concentration to examine the effect of TACI ligation on CD40 driven IgG1 and IgE synthesis. Fig. 1B shows that addition of APRIL

caused a significant ~ 2.5 fold increase of IgG_1 and IgE secretion by B cells treated with IL-4 and 75 ng/ml of anti-CD40. APRIL+IL-4 induced modest IgG_1 and IgE synthesis as previously described[1].

APRIL binds to BCMA and heparan sulfate proteoglycans (HSPGs) in addition to TACI[17, 18]. Either of these receptors could potentially mediate the enhancing effect of APRIL on CD40 driven Ig production by naïve B cells. Figure 2 shows that naïve B cells express TACI, but no detectable BCMA. CD40 ligation upregulated TACI expression, but had no detectable effect on BCMA expression after 72 hrs. This finding suggests that the effect of APRIL on CD40 activation of B cells was mediated via TACI.

To ascertain the role of TACI in APRIL mediated enhancement of CD40 activation, we examined the effect of anti-TACI antibody. Figure 3A shows that addition of goat anti-TACI enhanced CD40+IL-4 driven IgG_1 and IgE production by naïve B cells to a similar extent as APRIL. In contrast, control goat IgG had a negligible effect. Like APRIL, anti-TACI, in the presence of IL-4, induced IgG_1 and IgE production (a net increase of 30.3 ng/ml for IgG1 and of 43 ng/ml of IgE), confirming our previous finding that engagement of TACI causes CSR in naive B cells.

To exclude an autocrine mechanism by which engagement of TACI might induce expression of APRIL, which in turn might engage BCMA and HSPGs, we tested the ability of anti-TACI to increase Ig production induced by anti-CD40+IL-4 in B cells from $BCMA^{-/-}$ mice. In all experiments, B cells from WT control and $BCMA^{-/-}$ mice treated with anti-CD40+IL-4+ anti-TACI secreted higher amounts of IgG_1 and IgE than B cells treated with anti-CD40+IL-4 +control goat IgG. We observed variability between experiments in the amounts of IgG_1 and IgE produced by B cells from both $BCMA^{-/-}$ and WT controls, possibly due to the mixed background of these mice (C57/Bl6-129Sv). For this reason we have expressed the data as fold increase in Ig secretion. There was no significant difference in the increase of IgG_1 and IgE synthesis induced by TACI ligation between $BCMA^{-/-}$ mice and WT controls (Figure 3B). These results suggest that TACI mediates APRIL enhancement of Ig production induced by CD40. In addition, the use of anti-TACI eliminates potential contribution of BCMA or HSPGs that may occur with the use of APRIL.

TACI engagement does not increase B cell proliferation and survival induced by anti-CD40 and IL-4

CD40 driven CSR is thought to be division linked[19]. We tested the hypothesis that engagement of TACI increases the rate of division of CD40/IL-4 activated B cells. CFSE dye dilution analysis revealed that B cells stimulated with anti-CD40+IL-4 underwent eight division cycles in the presence of anti-TACI Ab versus nine cycles in the presence of control IgG (see Figure E1A in the Online Repository).

We also examined the effect of anti-TACI on the survival of B cells stimulated with anti-CD40. We used Annexin V staining to detect apoptotic cells at days 1, 2, 3, 4 and 6 after stimulation. As expected, the percentage of live cells was substantially higher in cells stimulated with anti-CD40+IL-4 compared to medium (see Figure E1B in the Online Repository). Addition of anti-TACI did not significantly affect the percentage or number of live cells. Altogether these data suggest that the increase in Ig production observed when both TACI and CD40 are engaged is neither due to clonal expansion nor to improved B cell survival.

TACI engagement has little effect on the induction of γ_1 germ line transcript (GLT) εGLT, Iμ-Cγ_1, Iμ-Cε and AID by anti-CD40 and IL-4

Molecular events involved in CSR include expression of GLTs and *AICDA*, followed by deletional switch recombination and expression of Iμ-C_H mature transcripts[20]. We tested the possibility that TACI signaling cooperates with CD40 and IL-4 signaling in inducing molecular events leading to CSR. As expected, anti-CD40+IL-4 induced γ_1GLT, εGLT and *AICDA* gene expression. There was only a slight increase in γ_1GLT, εGLT, Iμ-Cγ_1 Iμ-Cε, and *AICDA* mRNA expression upon addition of anti-TACI compared to goat IgG (Figure 4). Note that control goat IgG resulted in a non-specific increase in the γ_1GLT and Iμ-Cγ_1 signals.

TACI engagement increases the percentage of CD138$^+$, but not of surface (s) IgG_1^+, cells induced by anti-CD40 and IL-4

We next examined whether the increase in IgG_1 secretion induced by TACI ligation of naïve B cells stimulated with anti-CD40+IL-4 was associated with increased induction of $sIgG_1^+$ cells. Figure 5A (upper panels) and Figure 5B (left panel) show the results of a representative experiment and pooled results from 5 independent experiments in which unlabeled or CFSE labeled B cells were stained at day 6 for $sIgG_1$ expression. Addition of anti-TACI to cultures of B cells stimulated with anti-CD40+IL-4 caused no significant increase in $sIgG_1^+$ B cells compared to addition of control goat IgG. Anti-TACI+IL-4 caused no detectable increase in $sIgG_1$ expression, possibly because TACI by itself is a relatively weak inducer of IgG_1 switching. These results suggest that the addition of anti-TACI to anti-CD40 has a negligible effect on CSR to IgG_1.

In vitro stimulation with anti-CD40+IL-4 induces naïve B cells to differentiate into plasmablasts that secrete Igs in the culture supernatant and express on their surface syndecan-1/CD138. Figure 5A (lower panels) and Figure 5B (right panel) show the results of experiments in which the same B cells used to analyze $sIgG_1$ expression were examined for CD138 expression 6 days after stimulation. There was robust induction of CD138 expression in cultures stimulated with anti-CD40+IL-4 but little induction of CD138 expression by anti-TACI+IL-4, again possibly because TACI is a relatively weak inducer of isotype switching. Addition of anti-TACI, but not control goat IgG, to cultures stimulated with anti-CD40+IL-4 caused a significant increase in the percentage of CD138$^+$ cells compared to control goat IgG (13.71% versus 8.36%, p=0.034).

TACI engagement causes a modest increase in IRF4 expression in B cells stimulated with anti-CD40+IL-4

We examined the effect of anti-TACI on the expression of the plasma cell differentiation factors Blimp1 and IRF4 and on the expression of the cytokine IL-6 that delivers survival signals to bone marrow plasma cells[21]. Figure 6 shows that stimulation with anti-CD40+IL-4 and goat IgG for 3 days slightly increased IRF4 expression in naïve B cells and caused no detectable increase in Blimp1 expression. The same B cells strongly upregulated Blimp1 expression following LPS stimulation (43.9±27.2 fold induction, n=3). Addition of anti-TACI to anti-CD40+IL-4 caused a significant (p=0.028) increase in IRF4 mRNA expression compared to control goat IgG. In contrast, Blimp1 expression remained unaffected. Anti-TACI+IL-4 did not significantly upregulate either IRF4 or Blimp1 expression (data not shown). IL-6 mRNA expression was strongly upregulated by anti-CD40+IL-4. Addition of anti-TACI caused no further increase in IL-6 expression.

DISCUSSION

Our data indicate that TACI enhances the differentiation of B cells activated under limiting conditions of CD40 ligation into Ig secreting plasmablasts. This suggests that, in addition to its key role in the antibody response to T-independent antigens, TACI may be important for the antibody response to T-dependent antigens (TD).

We demonstrated that APRIL and anti-TACI enhanced IgG1 and IgE synthesis by naïve B cells stimulated with suboptimal concentrations of anti-CD40 and IL-4. In turn, CD40 stimulation upregulated TACI expression on B cells. Thus, there is crosstalk between TACI and CD40, because TACI ligation enhances Igs production induced by CD40 and CD40 ligation induces increased expression of TACI on the surface of B cells. Recently, it was reported that engagement of HSPG is important for APRIL-induced B-cell proliferation and production of IgA[22]. It is possible that TACI induction of CSR to IgA may have different signaling requirements than TACI enhancement of CD40 driven B cell activation.

Among the functions that we analyzed TACI and CD40 cooperation is limited to the Igs secretion. There was only a modest increase in the induction of the molecular events underlying CSR and no significant increase in the generation of $sIgG1^+$ switched B cells. Moreover, CD40-induced proliferation and viability of B cells was not affected by TACI engagement. In contrast, there was an increase in the percentage and number of $CD138^+$ plasmablasts that correlated with the increase in Ig secretion. These results suggest that TACI ligation exerted its effect on CD40 stimulated B cells post switching by inducing more B cells to differentiate to plasmablasts and/or possibly by enhancing the survival of plasmablats. While TACI appears to promote the generation of plasmablasts, BCMA is thought to be important for the survival of long-lived bone marrow plasma cells, but is not implicated in the generation of short-lived plasma cells[23]. Thus, TACI and BCMA may cooperate in inducing optimal Ig secretion.

The transcription factors Blimp1 and IRF4 play a critical role in plasma cell differentiation[4, 5, 24]. Although TACI ligation caused a robust increase in the generation of plasmablasts following CD40/IL-4 stimulation, it caused a modest, albeit significant, increase in IRF4 expression and no detectable increase in Blimp1 expression. This suggests that TACI may activate pathways, in addition to IRF4 and other than Blimp1, that promote plasma cell generation and/or survival. Potential candidates include Mcl-1, which is induced by TACI ligation and promotes the survival of B cell blasts and plasmablasts[25]. Alternatively, the modest increase observed in the levels of IRF4 might be sufficient to promote plasma cell differentiation.

The synergy we have observed between TACI ligation and CD40 may involve signaling pathways common to both receptors. These include recruitment of TNF receptor associated factor (TRAF) molecules and activation of MAPKs and of the transcription factors NF-kB and AP-1[26, 27], which have been shown to be important for CD40-induced CSR[14, 28]. In particular, TRAF6, which is engaged by both CD40 and TACI, is thought to be important for plasma cell differentiation[29]. Alternatively, TACI may recruit specific signaling intermediates, such as CAML, that synergize with pathways activated by CD40 to promote B cell differentiation.

Sakurai et al. recently reported that agonistic anti-TACI mAb attenuates proliferation and IgG secretion by human B cells activated by a relatively high concentration of sCD40L (2 mg/ml) [30]. This suggests that TACI ligation may have opposite effects on CD40 activation of B cells depending on the strength of the CD40 signal. These opposite effects could be mediated by PGE_2, the synthesis of which is induced by TACI ligation[25]. Low concentrations of PGE_2 enhance B cell differentiation and Ig production, while high concentrations of PGE_2 are inhibitory [25]. CD40L expression has been reported to be decreased in CVID patients[31]. Our

data suggest that impairment of TACI function could also contribute to the impaired antibody response to TD antigens in these patients. Although, it has been reported that TACI$^{-/-}$ mice mount a normal antibody response when immunized TD antigens using standard protocols[32]. Our data suggest that the response of these mice to suboptimal doses of TD antigen needs to be investigated.

Germinal centers (GCs) are a candidate location where B cells may receive signals via CD40, TACI and the B cell receptor (BCR). In GCs, B cells are in contact both with activated T cells that express CD40L and with dendritic cells which express APRIL and BAFF. Furthermore, ligation of the BCR, CD40 and TLR9 upregulates TACI expression on B cell[33] (Figure 2). TACI upregulation would be expected to further promote co-operation between TACI and CD40 in the response to TD antigens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by grants from the National Institutes of Health, AI031541 and AI031136 (RG), AI065762 (EC) and USIDNET, AI30070 (EC).

References

1. Castigli E, Wilson SA, Scott S, Dedeoglu F, Xu S, Lam KP, et al. TACI and BAFF-R mediate isotype switching in B cells. J Exp Med 2005;201:35–9. [PubMed: 15630136]
2. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822–9. [PubMed: 12154359]
3. He B, Qiao X, Cerutti A. CpG DNA induces IgG class switch DNA recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-10. J Immunol 2004;173:4479–91. [PubMed: 15383579]
4. Klein U, Casola S, Cattoretti G, Shen Q, Lia M, Mo T, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol 2006;7:773–82. [PubMed: 16767092]
5. Shapiro-Shelef M, Lin KI, McHeyzer-Williams LJ, Liao J, McHeyzer-Williams MG, Calame K. Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. Immunity 2003;19:607–20. [PubMed: 14563324]
6. Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM, et al. Plasma cell differentiation requires the transcription factor XBP-1. Nature 2001;412:300–7. [PubMed: 11460154]
7. Ohkubo Y, Arima M, Arguni E, Okada S, Yamashita K, Asari S, et al. A role for c-fos/activator protein 1 in B lymphocyte terminal differentiation. J Immunol 2005;174:7703–10. [PubMed: 15944271]
8. Gupta S, Jiang M, Anthony A, Pernis AB. Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4. J Exp Med 1999;190:1837–48. [PubMed: 10601358]
9. Castigli E, Wilson S, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet 2005;37:829–34. [PubMed: 16007086]
10. Salzer U, Chapel HM, Webster AD, Pan-Hammarstrom Q, Schmitt-Graeff A, Schlesier M, et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet 2005;37:820–8. [PubMed: 16007087]
11. Castigli E, Wilson S, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA. Nat Genet 2007;39:430–1. [PubMed: 17392798]
12. Cunningham-Rundles C, Ponda PP. Molecular defects in T- and B-cell primary immunodeficiency diseases. Nat Rev Immunol 2005;5:880–92. [PubMed: 16261175]

13. Xu S, Lam KP. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. Mol Cell Biol 2001;21:4067–74. [PubMed: 11359913]

14. Jabara H, Laouini D, Tsitsikov E, Mizoguchi E, Bhan A, Castigli E, et al. The binding site for TRAF2 and TRAF3 but not for TRAF6 is essential for CD40-mediated immunoglobulin class switching. Immunity 2002;17:265–76. [PubMed: 12354380]

15. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 2000;102:553–63. [PubMed: 11007474]

16. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45. [PubMed: 11328886]

17. Hendriks J, Planelles L, de Jong-Odding J, Hardenberg G, Pals S, Hahne M, et al. Heparan sulfate proteoglycan binding promotes APRIL-induced tumor cell proliferation. Cell Death Differ 2005;12:637–48. [PubMed: 15846369]

18. Ingold K, Zumsteg A, Tardivel A, Huard B, Steiner Q, Cachero T, et al. Identification of proteoglycans as the APRIL-specific binding partners. J Exp Med 2005;201:1375–83. [PubMed: 15851487]

19. Hasbold J, Corcoran LM, Tarlinton DM, Tangye SG, Hodgkin PD. Evidence from the generation of immunoglobulin G-secreting cells that stochastic mechanisms regulate lymphocyte differentiation. Nat Immunol 2004;5:55–63. [PubMed: 14647274]

20. Manis JP, Tian M, Alt FW. Mechanism and control of class-switch recombination. Trends Immunol 2002;23:31–9. [PubMed: 11801452]

21. Minges Wols HA, Underhill GH, Kansas GS, Witte PL. The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. J Immunol 2002;169:4213–21. [PubMed: 12370351]

22. Sakurai D, Hase H, Kanno Y, Kojima H, Okumura K, Kobata T. TACI regulates IgA production by APRIL in collaboration with HSPG. Blood 2007;109:2961–7. [PubMed: 17119122]

23. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. J Exp Med 2004;199:91–8. [PubMed: 14707116]

24. Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, Singh H. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. Immunity 2006;25:225–36. [PubMed: 16919487]

25. Mongini PK, Inman JK, Han H, Fattah RJ, Abramson SB, Attur M. APRIL and BAFF promote increased viability of replicating human B2 cells via mechanism involving cyclooxygenase 2. J Immunol 2006;176:6736–51. [PubMed: 16709833]

26. Ni C, Oganesyan G, Welsh K, Zhu X, Reed J, Satterthwait A, et al. Key molecular contacts promote recognition of the BAFF receptor by TNF receptor-associated factor 3: implications for intracellular signaling regulation. J Immunol 2004;173:7394–400. [PubMed: 15585864]

27. Xia XZ, Treanor J, Senaldi G, Khare SD, Boone T, Kelley M, et al. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. J Exp Med 2000;192:137–43. [PubMed: 10880535]

28. Jabara HH, Geha RS. Jun N-terminal kinase is essential for CD40-mediated IgE class switching in B cells. J Allergy Clin Immunol 2005;115:856–63. [PubMed: 15806010]

29. Ahonen C, Manning E, Erickson L, O'Connor B, Lind E, Pullen S, et al. The CD40-TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells. Nat Immunol 2002;3:451–6. [PubMed: 11967542]

30. Sakurai D, Kanno Y, Hase H, Kojima H, Okumura K, Kobata T. TACI attenuates antibody production costimulated by BAFF-R and CD40. Eur J Immunol 2007;37:110–8. [PubMed: 17154264]

31. Farrington M, Grosmaire L, Nonoyama S, Fischer S, Hollenbaugh D, Ledbetter J, et al. CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. Proc Natl Acad Sci U S A 1994;91:1099–103. [PubMed: 7508119]

32. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001;14:573–82. [PubMed: 11371359]

33. Ng LG, Ng CH, Woehl B, Sutherland AP, Huo J, Xu S, et al. BAFF costimulation of Toll-like receptor-activated B-1 cells. Eur J Immunol 2006;36:1837–46. [PubMed: 16791880]

ABBREVATIONS

APRIL
A proliferation inducing ligand

AICDA
activation induced cytidine deaminase

BAFF
B-cell activating factor of the TNF family

BCMA
B cell maturation antigen

Blimp1
B lymphocyte-induced maturation protein 1

CSR
class switch recombination

CVID
Common variable immunodeficiency

GC
germinal center

HSPGs
heparan sulfate proteoglycans

IRF4
Interferon regulatory factor 4

TACI
Transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor

TLR
Toll-like receptor

XBP-1
X-box binding protein 1

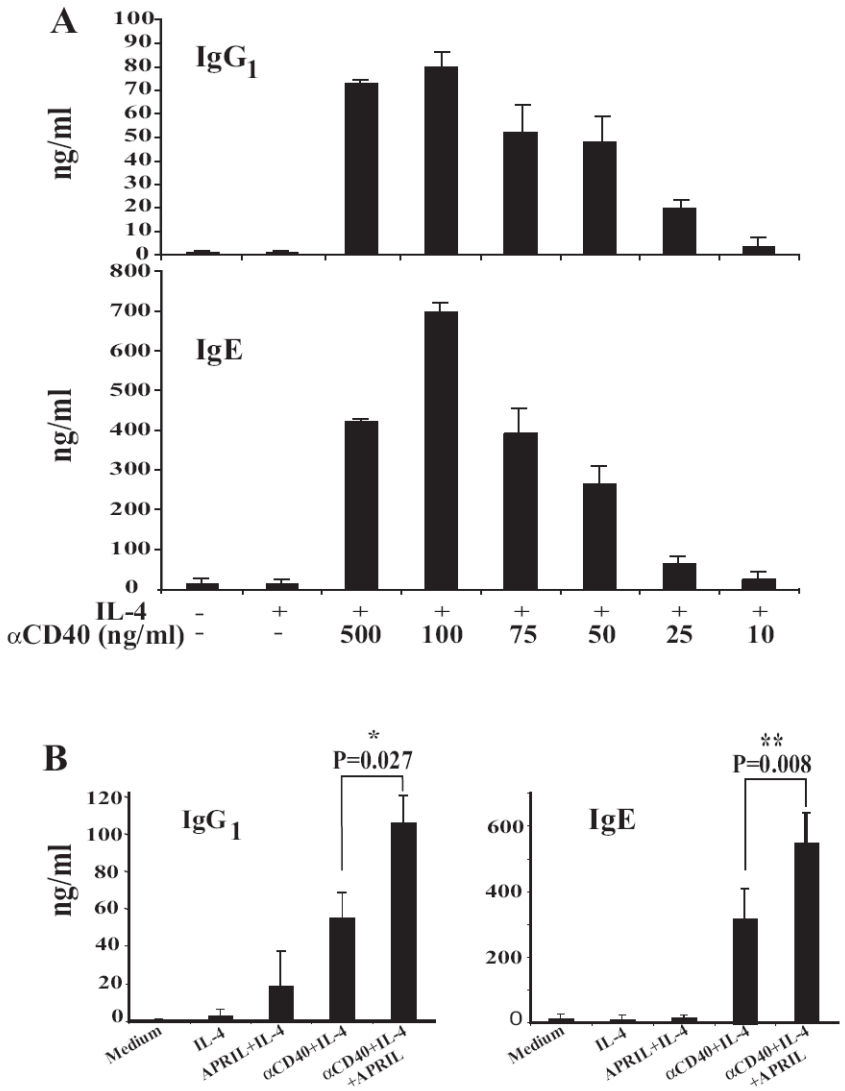

Figure 1. Effect of APRIL on IgG1 and IgE production induced by anti-CD40 and IL-4

A) IgG_1 and IgE production by naïve B cells in response to different concentrations of anti-CD40 and IL-4. **B).** Effect of APRIL on IgG_1 and IgE production driven by a suboptimal concentration of anti-CD40 (75 ng/ml). Bars represent the mean±S.E. of three (A) and four (B) independent experiments.

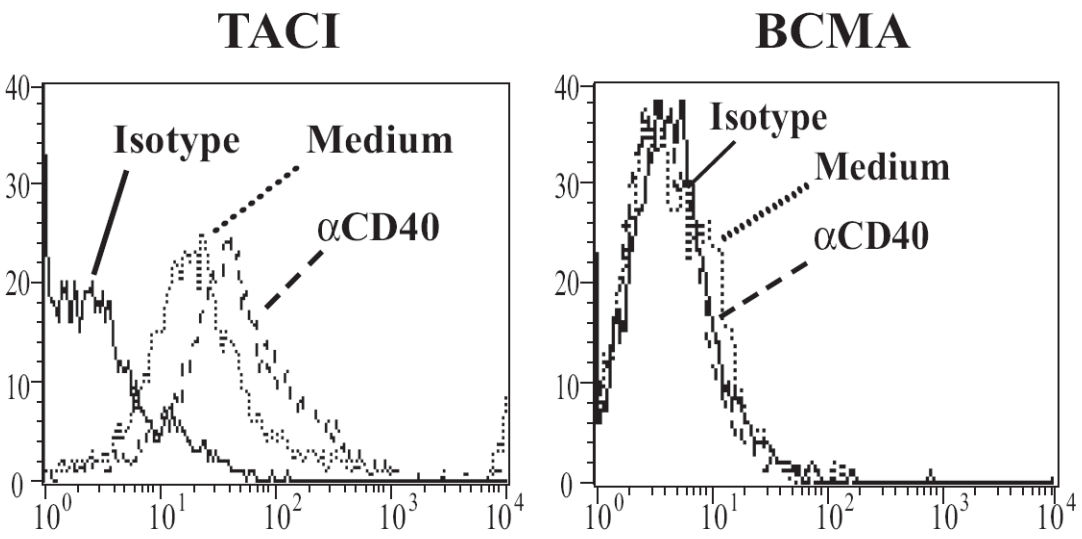

Figure 2. Effect of CD40 ligation on TACI expression

FACS analysis of TACI and BCMA expression in naïve mouse B cells before and after stimulation with anti-CD40 for 72 hrs. Similar results were obtained in 3 independent experiments.

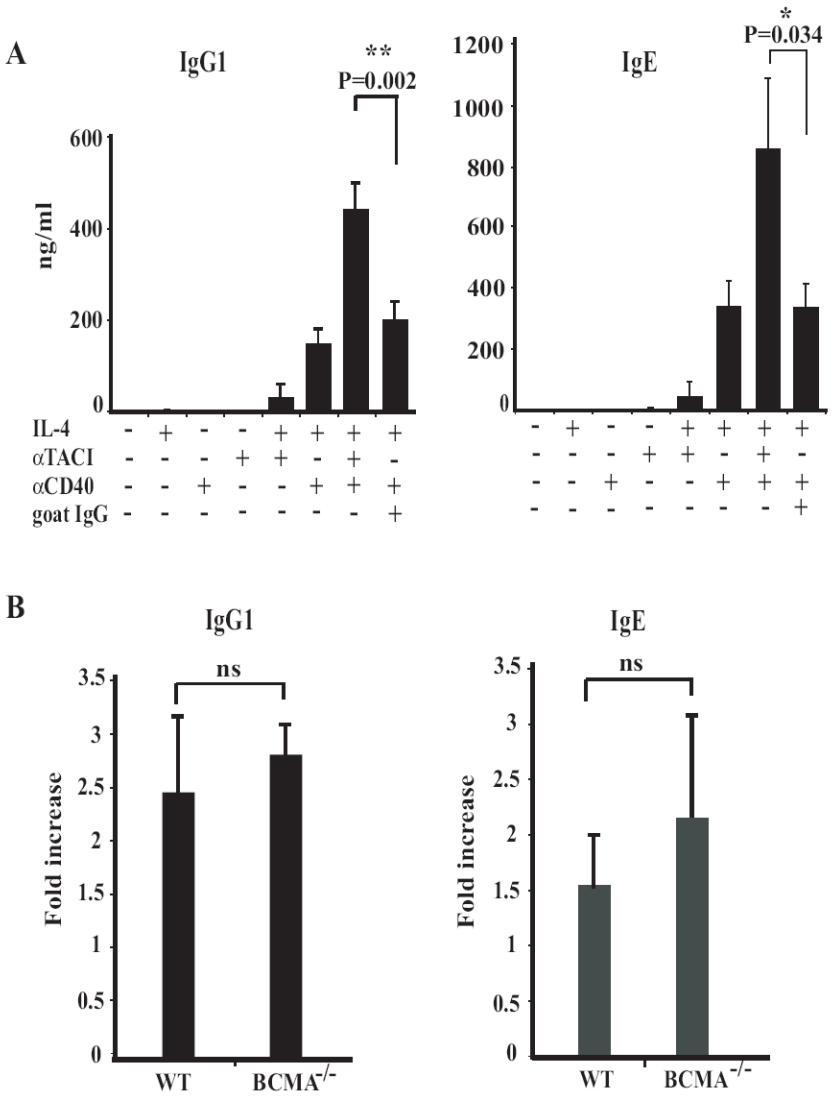

Figure 3. Effect of TACI ligation on IgG_1 and IgE production induced by anti-CD40 and IL-4

Effect of anti-TACI on IgG1 or IgE production in B cells from **(A)** BALB/c mice and from **(B)** $BCMA^{-/-}$ mice and WT controls. Bars represent mean±S.E. of independent five experiments in A and four in B.

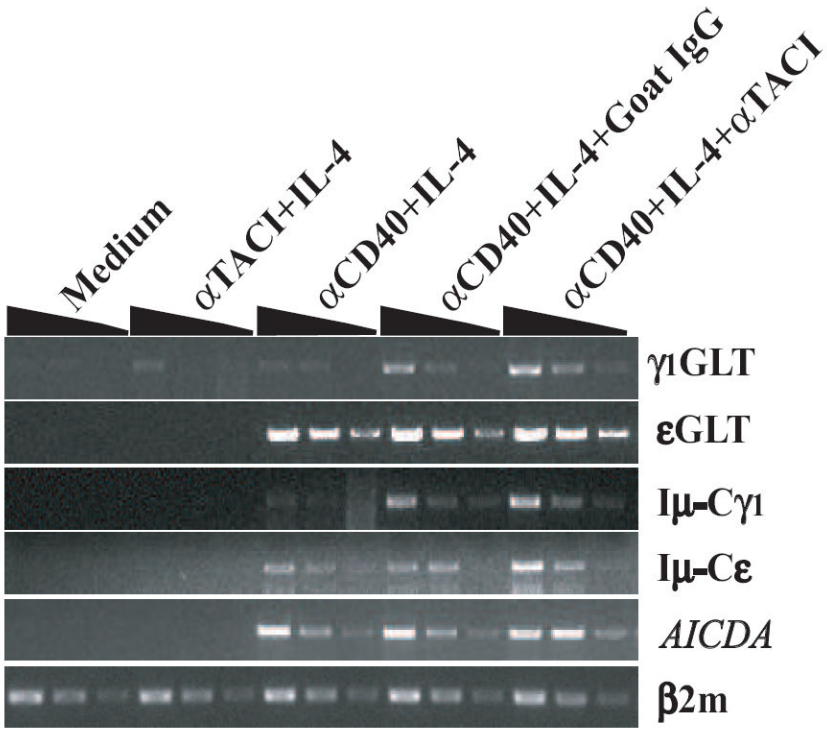

Figure 4. Effect of TACI ligation on molecular events involved in CSR induced by anti-CD40+IL-4

B cells were cultured for 3 days with the indicated stimuli. PCR was performed on cDNA samples diluted 1:1, 1:3, and 1:9. β_2m was used as loading control. Similar results were obtained in three independent experiments.

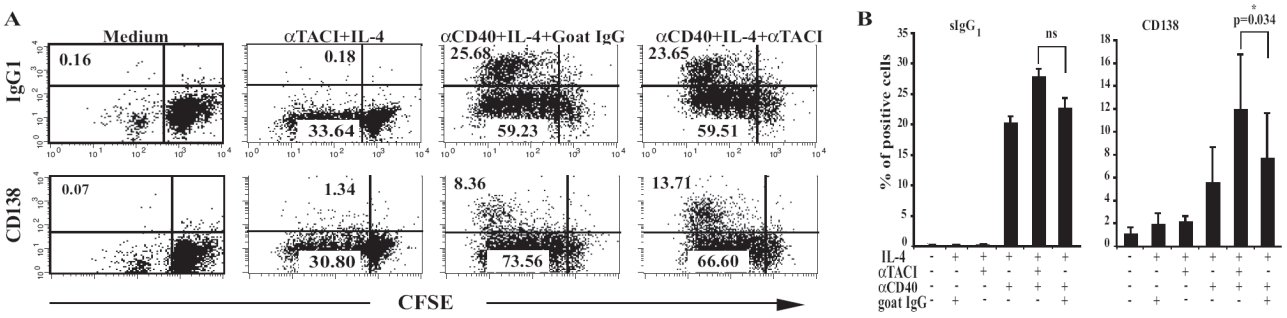

Figure 5. Effect of TACI ligation on IgG_1 and CD138 surface expression induced by anti-CD40 +IL-4

(**A**) B cells were loaded with CFSE and cultured for 6 days then analyzed by FACS for IgG_1 and CD138 surface expression. (**B**) Pooled results of 5 independent experiments. Bars represent mean±S.E.

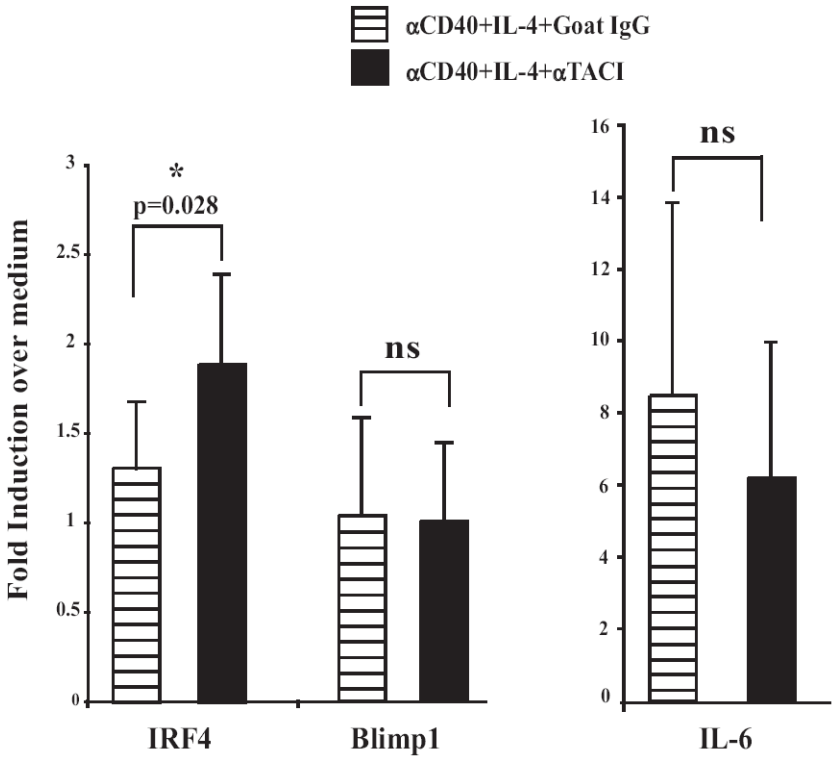

Figure 6. Effect of TACI ligation on the induction of IRF4, Blimp1 and IL-6 expression by anti-CD40+IL-4

Real time PCR on RNA from B cells cultured for 3 days with anti-D40+IL-4 and either goat IgG or goat anti-TACI. Bars represent the mean±S.E. of four independent experiments.