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Regulation of the T-Independent Humoral Response by TACI

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Summary

TACI is a TNFR homolog expressed by mature B lymphocytes that has been implicated in the positive regulation of B cell growth and antibody production, as well as in the development of autoimmune disease. Its biology is complex due to the existence of two ligands, BLyS and APRIL, and a homologous receptor, BCMA, that similarly binds both ligands. To determine its critical biological role, we generated TACI knockout mice. Surprisingly, these mice demonstrated a 2-fold increase in numbers of circulating and splenic B cells, apparently due to increased proliferation rate. Maturation of B cells and T-dependent antibody production was normal, but responses to T-independent type II antigens were almost completely abolished. It appears that TACI provides an essential costimulatory signal for the T-independent humoral response.

Introduction

Antibody production by B cells can be divided into T-dependent and T-independent types. The former encompasses the response to most complex proteins. An essential component of the T-dependent response includes the costimulatory molecule CD40, which resides on B cells and is activated by T cell-presented CD40L. This ensures appropriate T cell help, since CD40L is upregulated following stimulation of the T cell receptor (TCR) (Foy et al., 1996; Laman et al., 1996). Certain bacterial cell wall antigens, such as repeating polymers of carbohydrates, however, are unable to stimulate such T/B cell interaction and activation. Nature's answer to this deficiency appears in the form of a T-independent antibody production response that involves the stimulation of B cells by antigen-presenting dendritic cells (Fagarasan and Honjo, 2000). The details of the signals that regulate this capability are not completely understood, but the importance of this mechanism is well known since it contributes significantly to the eradication of bacterial infections. Involvement of this system in the development of some types of autoimmune disease (Fehr et al., 1997) further underscores the clinical importance for understanding the details of its regulation.

We previously cloned and characterized the gene encoding the transmembrane activator and CAML interactor (TACI) protein by virtue of its ability to physically interact with the CAML signal transduction protein. TACI appears to be a member of the tumor necrosis factor receptor (TNFR) family, based on limited sequence homology in its extracellular domain. It is expressed mainly on mature B cells and possibly is induced in a subset of T cells following their activation. Based on signaling studies indicating TACI's ability to activate the NF-κB, AP-1, and NF-AT transcription factors, we predicted a costimulatory role for TACI in some aspect of B cell growth or effector function; however, neither its true role nor its endogenous ligand was known at the time (von Bulow and Bram, 1997).

More recently, Gross et al. (2000) identified a ligand for TACI as the B cell growth factor zTNF4. ZTNF4 is a member of the tumor necrosis factor family and was previously identified by several groups (synonyms include BLyS [Moore et al., 1999], BAFF [Schneider et al., 1999], TALL-1 [Shu et al., 1999], THANK [Mukhopadhyay et al., 1999], and TNFSF13b). This finding was consistent with the proposed role for BLyS, since it binds to mature B cells and was known to selectively accelerate their growth. In addition, a distantly related TNFR family member, BCMA, was identified by Gross et al. (2000) as another B cell surface receptor able to bind to zTNF4. The ability of BLyS to bind to TACI and/or BCMA has independently been shown by several investigators (Marsters et al., 2000; Shu and Johnson, 2000; Thompson et al., 2000; Wu et al., 2000; Xia et al., 2000; Yan et al., 2000). Interestingly, it was noted that stimulation by BLyS, either in transgenic mice or by injection of recombinant protein, induced increased numbers of B cells and splenomegaly, as well as increased secretion of antibodies and the production of autoantibodies leading to symptoms of systemic lupus erythematosus (SLE) (Gross et al., 2000; Khare et al., 2000; Mackay et al., 1999). Conversely, the administration of soluble recombinant TACI protein into mice inhibited antibody production and the production of germinal centers in the spleen (Yan et al., 2000). Gross et al. found that spontaneously autoimmune mice developed higher levels of circulating BLyS, raising the possibility that enhanced stimulation of TACI or BCMA may contribute to the disease. Injection of spontaneously autoimmune mice with soluble recombinant TACI receptors suppressed kidney damage and increased survival (Gross et al., 2000), implicating a role for this set of signaling molecules in autoimmunity. More recently, others have also reported elevated serum concentrations of soluble BLyS in humans with SLE (Zhang et al., 2001), thereby highlighting the relevance of these mouse models to human forms of autoimmune disease.

The situation has been complicated by recent reports that indicate that another TNF family member, APRIL, is able to bind to both BCMA and TACI with similar affinity (Marsters et al., 2000; Wu et al., 2000). Both ligands (BLyS and APRIL) are able to compete with the other in binding to the TACI and BCMA receptors. In addition, there may be other, as yet undiscovered, ligands capable of binding to TACI and/or BCMA, or receptors for BLyS and APRIL. Thus, the precise contributions to humoral immunity by the individual members of this pathway are unclear. To more rigorously dissect the biological role of TACI in B cell production and function,

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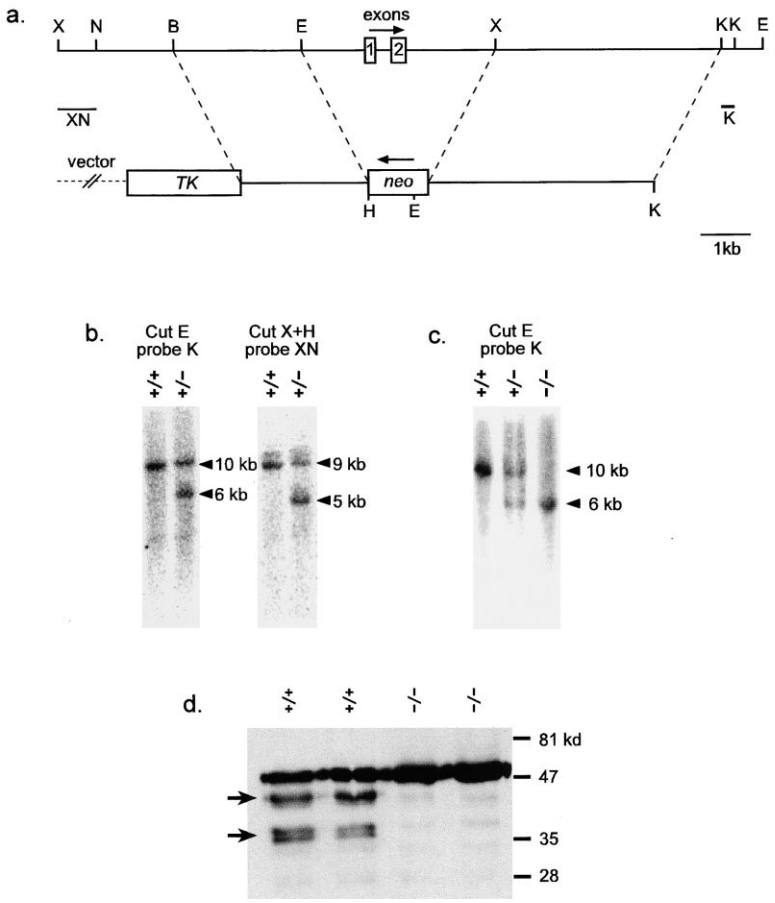

Figure 1. Targeting the *taci* Locus

(A) Targeting strategy for the *taci* locus showing the restriction map (top) and the targeting construct (bottom). Targeted exons 1 and 2 are boxed, and the direction of transcription is indicated with an arrow. Location of probes is indicated by XN and K. X, XmaI; N, NheI; B, BglII; E, EcoRI; K, KpnI; H, HindIII; TK, Thymidine kinase cassette; and neo, G418-resistance cassette.

(B) Southern blot of genomic DNA from +/+ and +/− ES cells showing the targeted allele. The left panel shows DNA from +/+ and +/− ES cells digested with EcoRI and hybridized with probe K. The right panel shows the same DNA digested with XmaI and HindIII and hybridized with probe XN.

(C) Southern blot of genomic DNA derived from wild-type, heterozygous, and taci knockout mice. Tail DNA was digested with EcoR1 and hybridized with probe K.

(D) Western blot demonstrating the deletion of the TACI protein from splenocytes. Spleens from two +/+ and −/− mice were extracted in detergent and immunoprecipitated with a rabbit polyclonal antibody against the extracellular domain of mouse TACI. The immunoprecipitates were then subjected to Western blotting and probing with the same anti-TACI antibody and peroxidase-linked goat anti-rabbit, followed by chemiluminescent detection (Amersham). The TACI-specific bands are indicated with an arrow. Cellular TACI protein frequently migrates on SDS-PAGE as doublets at 37 and 42 kDa. This likely represents incomplete reduction of the disulfide bridges, which make up the cysteine-rich domain of the receptor.

we have deleted the *taci* gene from mice and have found a requirement for the protein in T-independent antibody production and in the regulation of B cell numbers.

Results

Targeting the *taci* Locus

A genomic P1 clone containing the *taci* locus was obtained by PCR screening using oligonucleotides derived from the murine *taci* cDNA (von Bulow et al., 2000). A replacement targeting vector was constructed (Figure 1A), and one allele from 129SVJ embryonic stem (ES) cells was targeted as described in the Experimental Procedures section. Two strains of *taci*-targeted mice were derived from two independent ES clones and were maintained in parallel. In the results described below, similar data were obtained from each strain. TACI knockout mice are viable, fertile, and, when bred and stored in sterile housing for at least 6 months, have shown no external defects. The deletion of the TACI protein in the splenocytes of targeted mice was confirmed by immunoprecipitation followed by Western blot with a mouse TACI-specific polyclonal antibody (Figure 1D). A Mendelian distribution of genotypes was observed on average for all matings (data not shown). Furthermore, in all the data that follow, there was no significant difference between wild-type and $taci^{+/-}$ mice, indicating that a single *taci* allele is sufficient for function.

TACI null Mice Have Enlarged Spleens with Increased Numbers of Mature Circulating and Splenic B Cells

The spleens from immunologically naive TACI knockout mice were larger in size and mass when compared with those from their wild-type littermates (Figure 2A). Comparing the splenic lymphocytes, there were twice as many B cells in the TACI null spleens, whereas the T cell number remained the same (Figure 2B). Similarly, there were approximately twice the number of B cells present in peripheral blood of TACI knockout mice, whereas the numbers of CD4 and CD8 T cells cells did not signicantly differ when compared with littermate controls (Figure 2C). To assess whether this increase in B cell numbers in the TACI null mice was due to increased B cell proliferation, BrdU uptake into B220-positive splenocytes was determined 24 hr after intraperitoneal injection. Enhanced incorporation of BrdU into the TACI null splenic B cells (Figure 2D) indicates that there was increased turnover of these cells in the spleen, probably accounting for the increased B cell numbers observed both in the spleen and peripheral blood. These BrdU-positive cells were determined to be IgD-high, thereby excluding the possibility of germinal center cells contributing to the detected cycling population (data not shown). In the spleen, these cells appeared to accumulate as mature B cells (IgM-low, IgD-high) and were not arrested in the transitional or marginal zone stages (Figure 2E).

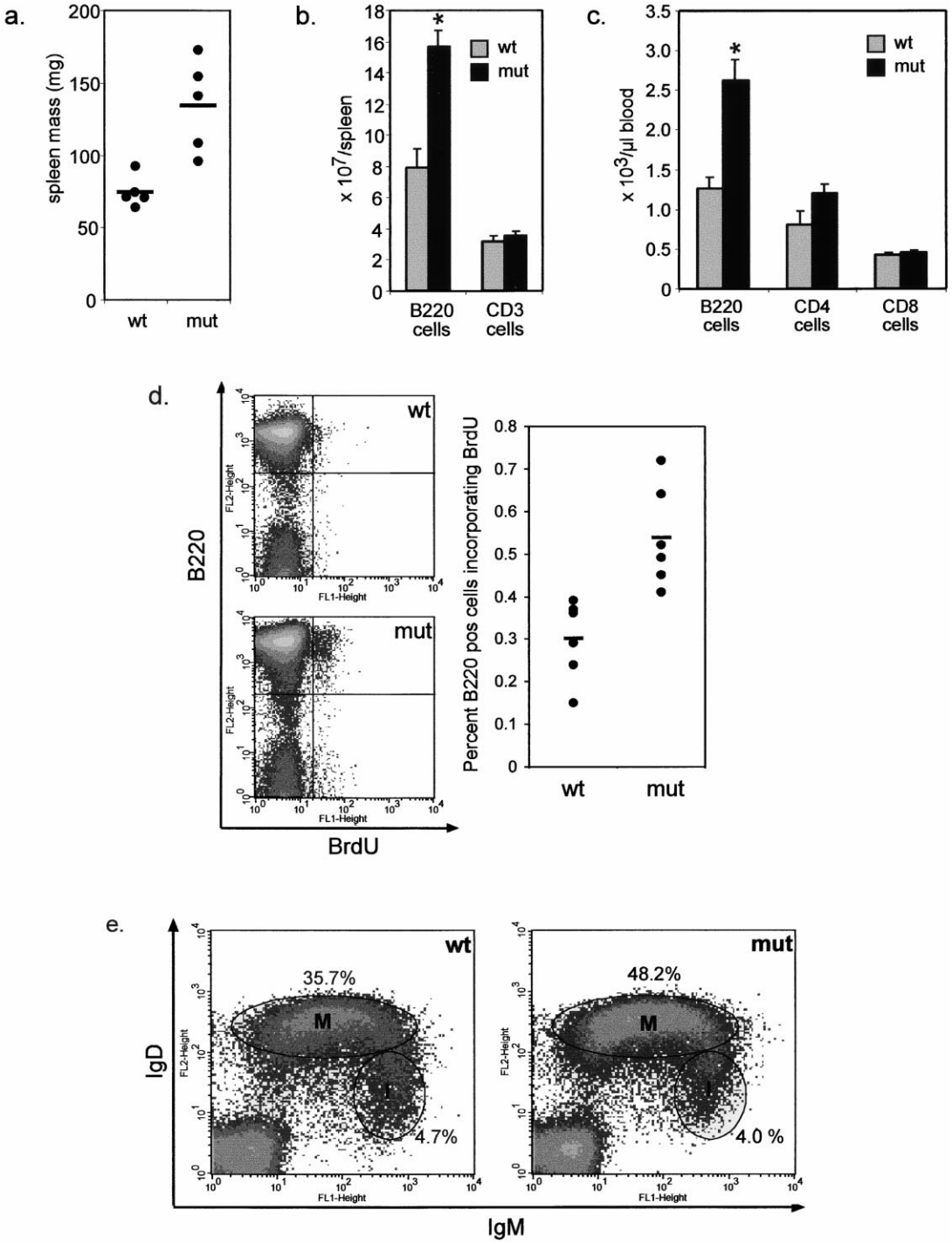

Figure 2. TACI null Mice Have Enlarged Spleens and Increased Numbers of Mature B Cells

(A) Spleen mass in TACI null mice and littermate controls; the bar represents the mean value.

(B) Absolute numbers of B (B220-pos.) and T cells (CD3-pos.) recovered from spleens of wild-type and TACI null mice. The relative proportion of B220 and CD3 cells was determined by flow cytometry and the numbers extrapolated from the total splenocyte count (N = 5, bars; SEM; *, $p < 0.01$).

(C) Absolute numbers of B and T lymphocytes in fresh blood from TACI null mice and littermate controls. The relative proportion of B220, CD4, and CD8 cells was determined by flow cytometry and the numbers extrapolated from the lymphocyte value obtained from a full blood count (N = 5, bars; SEM; *, $p < 0.01$).

(D) Cell cycle turnover in splenocytes from naive mice. Mice were injected with BrdU 24 hr before sacrifice. Splenocytes were prepared, stained with phycoerythrin-conjugated anti-B220 and FITC-conjugated anti-BrdU, followed by flow cytometry. Gates were set on forward and side scatter as well as the B220/BrdU-positive events. Representative density plots for wild-type and TACI null mice are depicted at left. Axes represent log fluorescence intensity. The proportions of B220-positive cells, which had incorporated BrdU within 24 hr from each mouse, are depicted at right. The bar represents the mean of six mice in each cohort.

(E) Flow cytometric analysis demonstrating that the majority of B cells in spleens from TACI null mice are mature. Splenocytes were prepared from naive normal and TACI null mice, stained with phycoerythrin-conjugated anti-IgM and FITC-conjugated anti-IgD, and then subjected to flow cytometry. Depicted are data from a representative control (left) and TACI null (right) mouse from a cohort of five each. Immature B cells and marginal zone cells are IgD-low and IgM-high (I), whereas the mature B cells are IgD-high and IgM-low (M). It can be seen for both control and TACI null mice that the majority of splenic B cells are mature.

TACI null Splenocytes Bind to and Show a Proliferative Response to BlyS

Since the TACI knockout mice showed increased numbers of B cells, a phenotype paradoxically similar to that of BLyS overexpression observed by others, we predicted that TACI null splenocytes would proliferate normally in response to BLyS. Tritiated thymidine uptakes were determined in TACI null and control splenocytes stimulated in vitro with recombinant human BLyS in the presence of IL-4 and crosslinking anti-IgM antibodies. The cells from TACI null mice were able to respond to BLyS, and no significant difference was observed when compared with cells from littermate controls (Figure 3A). Binding of epitope-tagged recombinant BLyS to B220-positive splenocytes, determined by flow cytometry (Figure 3B), showed no difference in binding between B cells from TACI null mice and their wild-type littermate controls. Binding of BLyS on splenocytes is likely due to BCMA, which probably mediates the proliferative response to the ligand.

TACI null Mice Exhibit Normal T-Dependent Humoral Responses

Comparison of serum antibody titers from naive normal and TACI mutant mice revealed a significant decrease in IgM and IgA concentrations, although total IgG concentrations were comparable (Figure 4A). There were no differences in the IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3 isotypes (data not shown), implying that the TACI null B cells can undergo normal isotype switching. Following immunization with the T-dependent antigen, keyhole limpet hemocyanin (KLH), both TACI mutant and normal mice responded similarly, with KLH-specific IgM being secreted by day 4 and IgG switching occurring by day 8. Serum IgG concentrations increased and IgM concentrations decreased with time (Figure 4B). Similar results were obtained with another T-dependent antigen, NP-conjugated chicken γ-globulin (NP-CgG) (data not shown). We conclude that the T-dependent humoral response is not impaired in TACI null mice.

The T-Independent Type II Humoral Response Is Defective in *TACI* null Mice

Immunization with the T-independent type I antigen, NP-conjugated lipopolysaccharide, showed no significant difference in specific antibody secretion by TACI mutant and wild-type mice (data not shown). However, when mice were immunized with the T-independent type II (TI-II) antigen NP-ficoll, we observed a significant defect in secretion of both specific IgM and IgG in the TACI mutant mice in comparison with littermate controls (Figure 5A). NP-specific IgM secretion in the mutant mice was about one-third, and specific IgG secretion was about one-fifth, of the amount produced by littermate controls. This decrease in secretion was detected for all the IgG isotypes tested (Figure 5B). Increasing the dose of NP-ficoll 10-fold to 50 μg per animal, as well as reducing it to 1μg, did not overcome the defect in reponse by TACI null mice (data not shown). Deletion of the *taci* gene induced a loss of 80% of the specific antibody response to a second T-independent antigen, Pneumovax, in comparison with their littermate controls (Figure 5C).

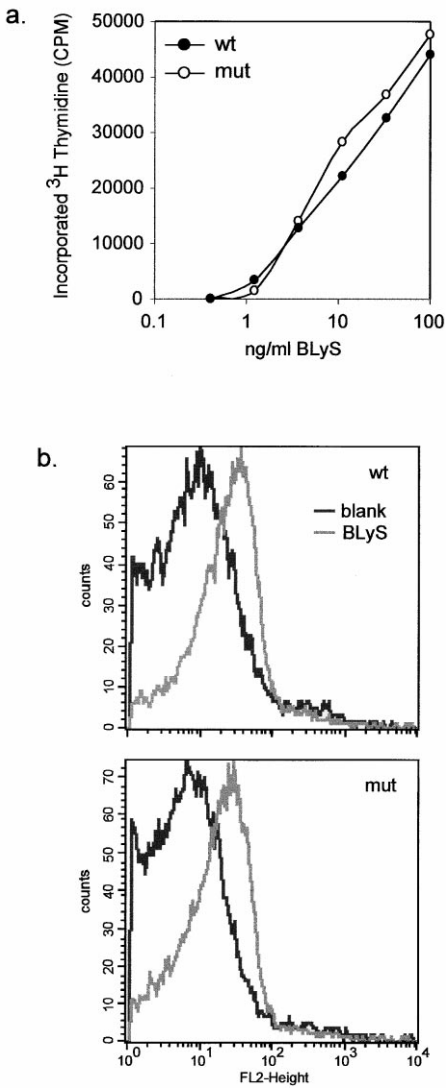

Figure 3. TACI null Splenic B Cells Bind and Proliferate in Response to BlyS

(A) Proliferative response to BLyS. Splenocytes were prepared from TACI null and littermate control mice. Cells were incubated for 72 hr with the indicated concentrations of human recombinant BLyS in the presence of murine IL-4 and crosslinking goat anti-mouse IgM. Tritiated thymidine incorporation was determined over the last 24 hr. Depicted data are from individual mice and are representative of five animals in each cohort. Background counts in response to anti-IgM and IL-4 alone ranged from near 4000 to 6000 CPM within each cohort of mice and were subtracted from the values depicted. (B) Flow cytometric analysis of BLyS binding to splenic B cells. After blocking Fc receptors, splenocytes were incubated in the presence (gray line) and absence (black line) of soluble Flag epitope-tagged human recombinant BLyS (0.1 μg/10^6 cells). BLyS binding was detected with biotinylated anti-Flag M2 antibodies followed by incubations with phycoerythrin-conjugated streptavidin. Depicted are representative histograms showing BLyS staining obtained from normal (top panel) and TACI null (bottom panel) B220-positive splenocytes (FL2-height, log fluorescence).

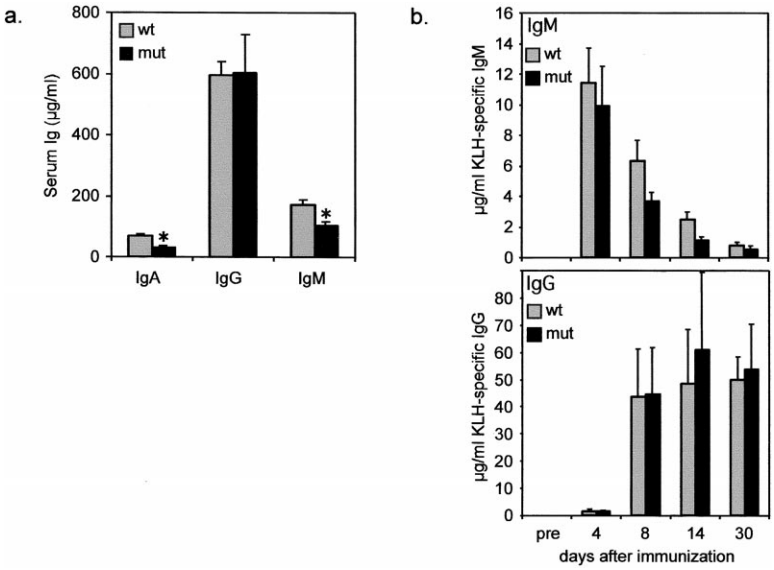

Figure 4. T-Dependent Humoral Responses Are Normal in TACI null Mice

(A) Total serum immunoglobulins from 6-week-old TACI null mice and littermate controls measured by ELISA (n = 5; *, P < 0.01).

(B) Humoral response to KLH, a T-dependent antigen. Cohorts (n = 5) of TACI null and littermate control mice were immunized with 50 μg KLH, and blood was sampled on the days indicated. Concentrations of KLH-specific serum IgM (top panel) and IgG (bottom panel) were detected by ELISA.

Splenic Architecture in *TACI* null Mice

The different types of antibody responses occur in geographically distinct compartments within the spleen. To further investigate a possible failure of normal B cell migration within the spleens of TACI null mice, we examined splenic architecture. In spite of spleen enlargement, with an increased B cell component in TACI null mice, the architecture of these spleens appeared normal. Immunofluorescent staining of spleen sections with antibodies to B220 and CD3 indicated a similar arrangement of T and B cells in the follicles when comparing wild-type and TACI null spleens (Figure 6A). Staining of sections with IgM and IgD (Figure 6B) also showed similar arrangement of mature (IgD-high) B cells and marginal zones (IgM-high). Further analysis of the marginal zone (MZ) revealed the presence of MAdCAM-1-positive endothelial reticular cells forming the follicular boundary as well as CD11c-positive dendritic cells, providing confirmatory evidence that the MZ architecture is normal in the TACI null mice and contains a myeloid component necessary for antigen presentation (Figure 6C). Furthermore, spleen sections from TACI null mice immunized with a T-dependent antigen, NP-conjugated chicken γ-globulin, showed normal germinal center formation as evidenced by staining with peanut lectin (Figure 6D). Others have demonstrated that B1 and marginal zone B (MZB) cells are essential for the TI-II humoral response (Prior et al., 1994; Scher, 1982; Guinamard et al., 2000). Since TACI null mice failed to respond normally to TI-II antigens, we searched for the presence of these cells in our mice. Relative numbers of B1 cells (B220- and CD5-positive) were determined in blood, spleen, and peritoneum, and no significant differences were observed between cells from TACI null mice and their normal littermates (Figure 7A). Marginal zone B cells (B220-positive, CRI/CRII-positive, and CD23-negative) were

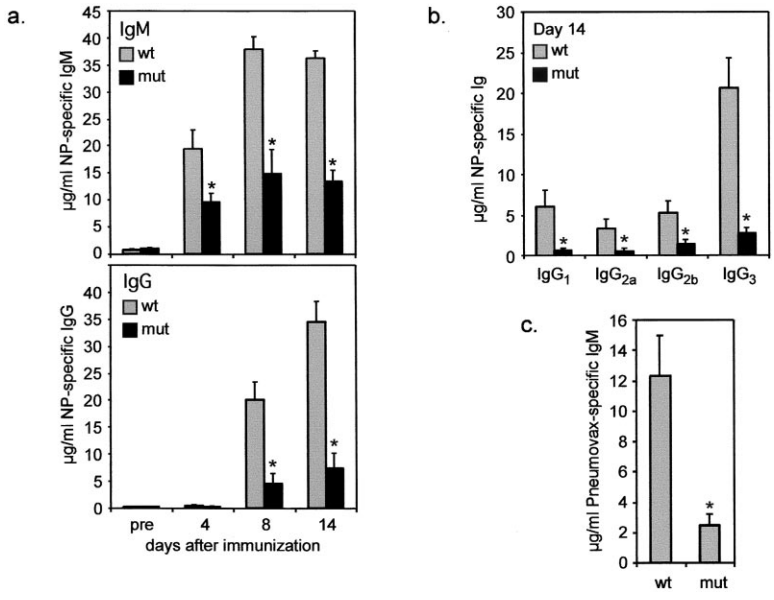

Figure 5. The T-Independent Type II Humoral Response Is Defective in TACI null Mice

(A) Humoral response to NP-ficoll, a T-independent type II antigen. Cohorts (n = 5) of TACI null and littermate control mice were immunized with 5 μg NP-ficoll, and blood was sampled on the dates indicated. Concentrations of NP-specific IgM (top panel) and IgG (bottom panel) were determined by ELISA (*, p < 0.01).

(B) Distribution of IgG isotypes in sera from TACI null and normal littermate mice after immunization with NP-ficoll. IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3 concentrations were determined by ELISA from the day 14 sera of NP-ficoll-immunized mice depicted in (A) (*, P < 0.01).

(C) IgM response to Pneumovax. Cohorts (n = 5) of TACI mutant mice and littermate controls were immunized with Pneumovax, and Pneumovax-specific serum IgM was determined after a week.

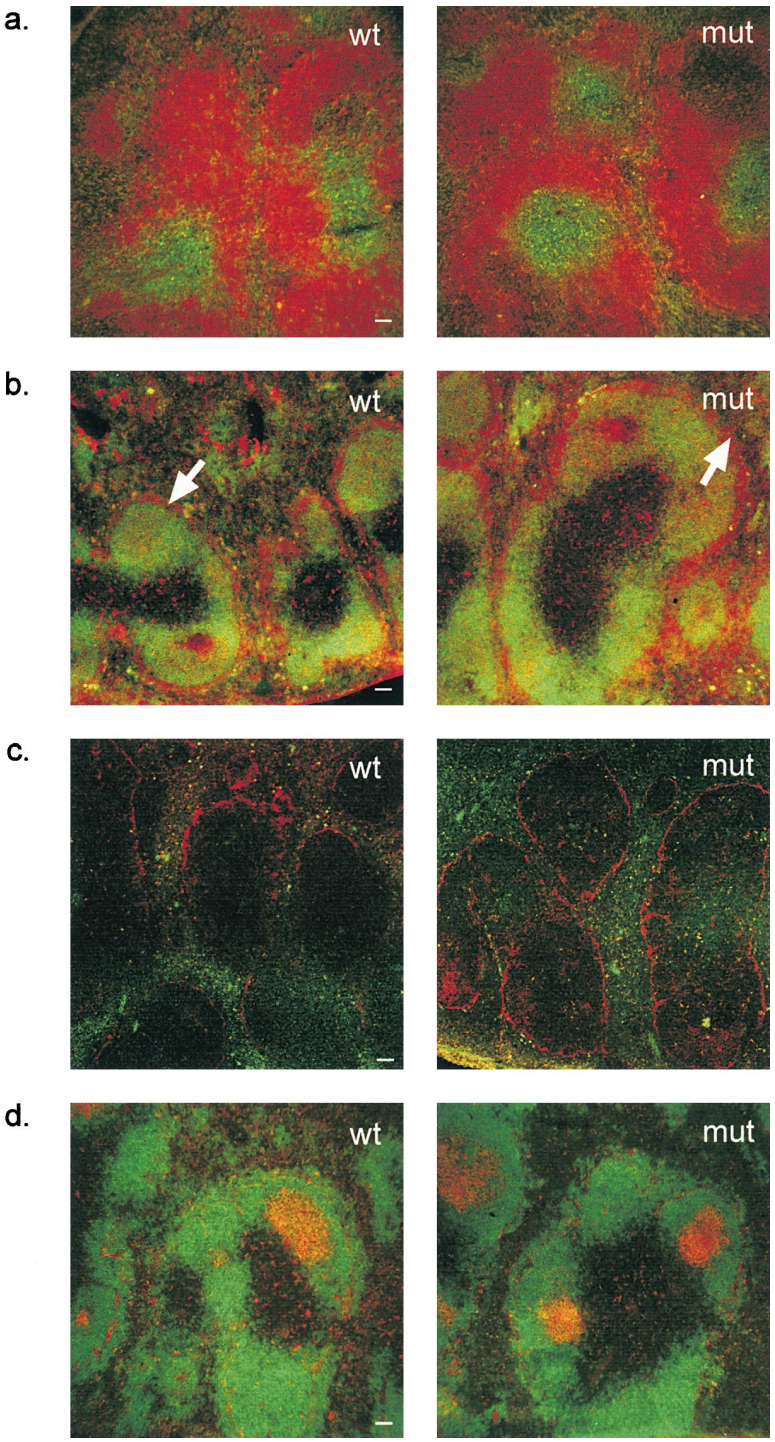

Figure 6. The Splenic Architecture of TACI null Mice Is Normal

Confocal images showing immunofluorescent staining of wild-type (wt) and TACI mutant (mut) spleen sections from naive mice (A–C).

(A) Distribution of B and T cells. Staining for B cells was with biotinylated anti-B220, followed with Texas Red-conjugated streptavidin, for T cells with FITC-conjugated anti-CD3. The white bars represent 10 μm.

(B) Marginal zone (arrows) and mature B cell distribution. Sections were stained with biotinylated anti-IgM, followed with Texas Red-streptavidin, and FITC-conjugated anti-IgD.

(C) Marginal zone architecture showing the MZ reticular endothelial cells and CD11c-positive dendritic cells. Sections were stained with anti-MAdCAM-1, followed with rhodamine-conjugated goat anti-rat IgG and FITC-conjugated anti-CD11c.

(D) Germinal center formation. Mice were immunized with alum-precipitated chicken γ-globulin (50 μg). The animals were sacrificed after 8 days and spleen sections were stained with biotinylated peanut agglutinin followed by Texas Red-streptavidin and counterstained with FITC-conjugated anti-B220.

also detected in similar proportions in spleens from normal and TACI null mice (Figure 7B). This indicates that the cells required for a TI-II response are present in TACI null mice.

Discussion

This report describes the first member of the TACI/BLyS receptor-ligand family to be deleted in mice. We find that TACI null mice have increased numbers of B cells, in both spleen and peripheral circulation. This result was unexpected because others have shown that BLyS, a known ligand for both TACI and the homologous TNFR member BCMA, induces B cell proliferation. The simplest explanation for these results is that TACI may ordinarily induce a growth inhibitory signal that is offset by a proliferative signal induced by BCMA. Although both receptors are thought to activate NF-κB, there is no

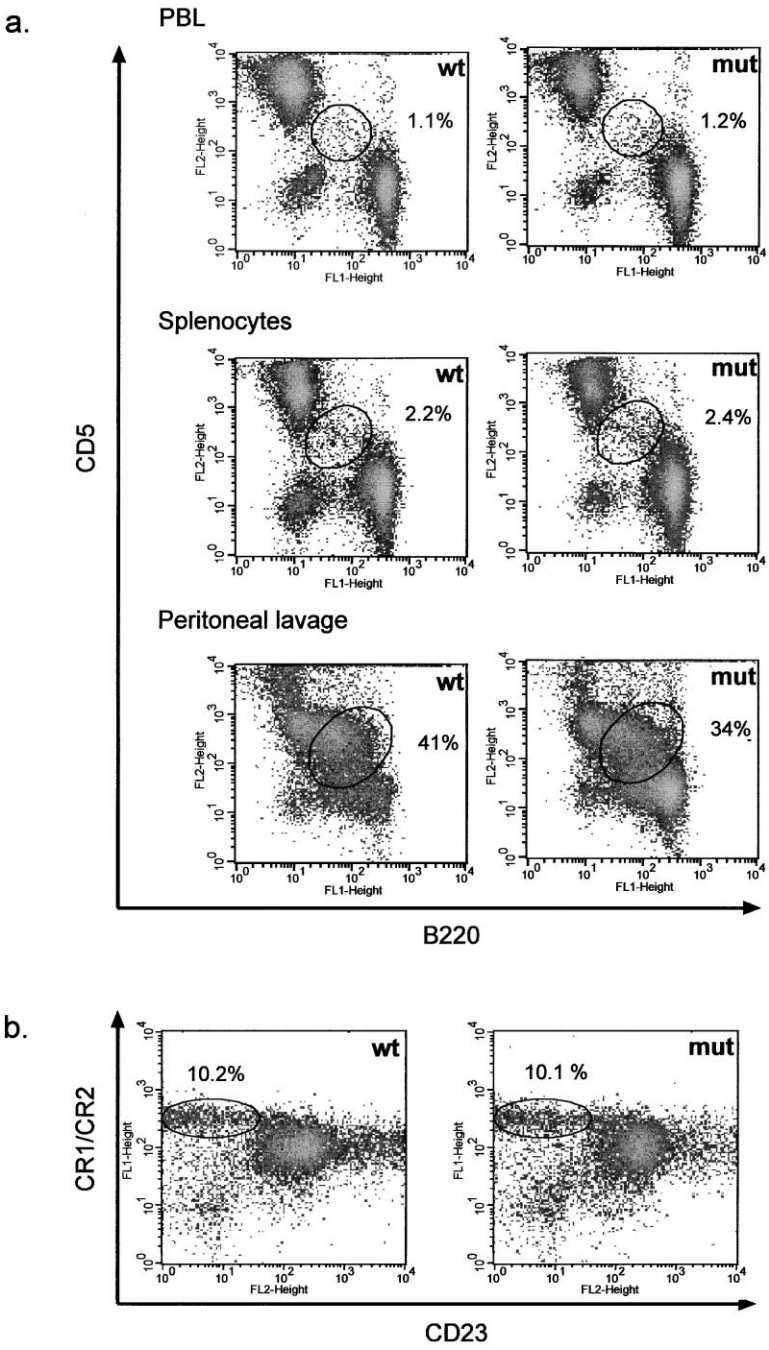

Figure 7. TACI null and Wild-Type Mice Have Similar Proportions of B1 and Marginal Zone B Cells

Depicted are representative results from four mice in each cohort; differences are not statistically significant. (A) Flow cytometric analysis of B1 cells (B220 pos. and CD5 pos.) derived from peripheral blood (PBL), splenocytes, and peritoneum. (B) Flow cytometric analysis of marginal zone cells from IgM-high gated splenocytes. The MZ cell population is B220-positive, CR1/CD2-high, and CD23-negative, as indicated.

sequence homology within their intracellular domains and there likely exist other second messengers that are differentially activated by each. According to this model, B cell proliferation in TACI null mice is enhanced because of increased stimulation through BCMA, in the absence of its competitor, TACI. This notion will be addressed in the future by the generation of BCMA knockout mice.

Mice bearing a BLyS transgene show increased B cell numbers with an enriched population of B cells in the spleen that accumulate at an immature stage of development (Batten et al., 2000). Our mice demonstrated normal maturation of B cells, thus indicating that B cell maturation is not critically dependent upon TACI. We cannot rule out at this point, however, a potential role for TACI in B cell proliferation that can be redundantly provided by BCMA in this knockout mouse.

We have demonstrated a requirement for TACI in order to mount a robust TI-II antibody response, whereas the T-dependent response does not depend on TACI. This complements work by others showing that both CD40 (Castigli et al., 1994) and CD40L (Xu et al., 1994) knockout mice have defective T-dependent humoral immunity but normal responses to T-independent antigens. We hypothesize that these selective defects are a function of the splenic architecture and access of B cells to

CD40L-bearing T cells. The T-dependent response takes place within splenic follicles, facilitating B cell access to T cells of the periarteriolar lymphoid sheath (PALS), which is intact in *taci*$^{-/-}$ spleens. T cells provide costimulatory signals such as CD40L, which are required for the B cell humoral response to T-dependent antigens. On the other hand, B cells responding to T-independent type II antigens are largely extrafollicular, residing in the marginal zone. In the absence of a CD40 signal, these cells appear to require a TACI signal to mount a robust response. In the marginal zone, antigen is presented directly to B cells by dendritic cells (Banchereau and Steinman, 1998), and a costimulatory role for BLyS (which is itself expressed by dendritic cells) in this function has been suggested (Laabi and Strasser, 2000). Dendritic cells genetically modified to express CD40L have been shown to activate humoral immunity in the absence of T cells (Kikuchi et al., 2000). Furthermore, it has been demonstrated in mice that the ordinarily poor humoral response to polysaccharides from *S. pneumoniae* can be augmented by administering antibodies that crosslink CD40, thereby mimicking T cells in their absence (Dullforce et al., 1998). Since deletion of the TACI protein has no effect on the T-dependent humoral responses, it seems that CD40L presented by T cells fulfills the costimulatory requirement and thereby allows B cell activation. We propose therefore that TACI is a functional homolog of CD40 able to costimulate B cells in a similar manner in the absence of T cells in the marginal zone. There are other parallels between CD40 and TACI. Both are members of the TNFR family and signal through binding of TRAF proteins to activate the NF-κB transcription factors (Baker and Reddy, 1998; von Bulow and Bram, 1997; Xia et al., 2000).

T-dependent humoral responses in the TACI null mice appeared normal. TACI null mice produced IgM in response to KLH and CgG, demonstrated normal isotype switching to produce IgG, and germinal center formation in the spleen. Numbers of T cells in peripheral blood and in the spleen were also normal. Additionally, mixed lymphocyte cultures showed a standard level of proliferation of T cells derived from TACI null mice upon stimulation with irradiated allogeneic lymphocytes (data not shown), indicating normal functioning of T cells responding through MHC class II receptors. Although we previously observed TACI-specific staining of a subset of in vitro-activated human peripheral T cells, it appears that the deletion of TACI in mice has no critical effect on T-dependent functions in vivo.

Other knockout models that have led to a defective TI-II response include mice lacking tyrosine kinase Pyk-2 (Guinamard et al., 2000), Brutons tyrosine kinase (Btk) (Prior et al., 1994; Scher, 1982), and phospholipase C-γ2 (Wang et al., 2000). The *pyk2*$^{-/-}$ mice lack marginal zone B cells, whereas the *btk*$^{-/-}$ mice lack B1 cells, demonstrating that MZB and B1 cells are required for a TI-II response. The *PLC*$\gamma_2{}^{-/-}$ mice also have a B1 cell deficiency and a block in B cell maturation at the pro-B stage. In contrast, TACI knockout mice have mature B cells, MZB cells, and B1 cells, suggesting that, since the required cells for a TI-II response are in place, the humoral defect lies with the response of these cells. We believe the development of this mouse model will help to elucidate molecular events that contribute to an effective antibody response and may also provide a useful animal model of immunodeficiency conditions in humans in which B cells do not function appropriately in response to TI-II antigens.

Experimental Procedures

The Mayo Institutional Animal Care and Use Committee approved all animal protocols.

Targeting the *taci* Locus

A murine P1 library (Genome Systems) was screened by PCR with synthetic deoxyoligonucleotides (5′-GGAAACGTGTAGCTTCTGC-3′ and 5′-GTCATTCTCCATCTTTCTCGA-3′) amplifying a 415 bp exon fragment from murine *taci* cDNA (von Bulow et al., 2000). The resulting P1 clone was mapped by Southern blot using murine *taci* cDNA fragments as probes. A targeting construct was cloned into pBluescript (Stratagene) in which an ~4 kb fragment containing the first two exons (encoding the extracellular domain) of *taci* was replaced with a *neo* cassette, and an *HSV-TK* cassette was inserted 3.5 kb upstream of the targeted sequence (Figure 1A). The construct was linearized and electroporated into murine embryonic stem (ES) cells from 129SVJ mice (Genome Systems). ES cells were maintained and drug selected on murine embryonic fibroblasts as described (van Deursen et al., 1996). Drug-resistant ES clones were screened for homologous recombination by Southern blot (Figure 1B). Correctly targeted ES clones were karyotyped, screened against mycoplasma, and injected into C57Bl/6 blastocysts (van Deursen et al., 1993), which were transferred to pseudopregnant foster mothers to yield chimeric mice. Chimeric males were crossed with C57Bl/6 females, and agouti F1 offspring were screened for the targeted *taci* allele by PCR. Homozygous targeted mice were derived by crossing F1 heterozygous mice and screening by PCR and/or Southern blot (Figure 1C). The genetic background of the resulting colony was equally 129SVJ and C57Bl/6. Deletion of the TACI protein was confirmed by immunoprecipitation from splenocyte lysates with mTACI-specific polyclonal antibodies, followed by Western blot with the same antibodies.

Immunizations

Mice (8 to 12 weeks of age) were inoculated intraperitoneally with the following antigens diluted in phosphate buffered saline (PBS): (4-hydroxy-nitrophenacetyl)-conjugated chicken γ-globulin (NP-CgG, Biosearch Technologies), keyhole limpet hemocyanin (KLH, Calbiochem), NP-conjugated lipopolysaccharide from *E. coli* (NP-LPS, Biosearch Technologies), NP-conjugated ficoll (NP-ficoll, Biosearch Technologies), and a collection of capsular polysaccharide preparations from 23 strains of *S. pneumoniae* (Pneumovax, Merck).

ELISAs

High-capacity ELISA plates (Maxsorb, Nunc) were coated with 10 μg/ml goat anti-Ig (Southern Biotechnologies) for assay of total serum antibodies and standards; 10 μg/ml NP-conjugated bovine serum albumin (NP_{40}-BSA, Biosearch Technologies) for the assay of NP-specific haptens; both in 50 mM carbonate/bicarbonate (pH 9.2) for 1 hr at 37°C; or 1 μg/ml Pneumovax in PBS overnight at 4°C for the assay of pneumococcus vaccine-specific antibodies. Plates were blocked with 1% gelatin in PBS for 1 hr at room temperature, washed with PBS + 0.05% Tween 20 (PBS-T), and loaded with sera serially diluted with 0.1% gelatin in PBS. After 30 min incubation at 37°C, plates were washed with PBS-T and incubated with alkaline phosphatase-conjugated goat anti-mouse IgM, -total IgG, -IgG isotypes, or -IgA (Southern Biotechnologies). Following washes with PBS-T, the substrate solution (80 mM 4-methylumbelliferyl phosphate in 1 M diethyl amine [pH 10]) was added and read on a fluorescent plate reader (Wallac) after a 30 min incubation at room temperature. Data were quantified against appropriate purified mouse immunoglobulin standards (Southern Biotechnologies).

Flow Cytometry

Heparinized whole blood (100 μl) was treated with Fc-block (Pharmingen-Becton Dickinson) and incubated sequentially with the relevant fluorochrome-conjugated antibodies (unless otherwise stated,

all from Pharmingen-Becton Dickinson) at 0.5μg /100 μl in Hanks Balanced Salt Solution (HBSS) with 1% BSA. After the final staining step, red blood cells were lysed and lymphocytes fixed with "FACS lysis solution" (Becton Dickinson). Cells were analyzed on FACScan or FACScalibur flow cytometers using the "Cellquest" software package (Becton Dickinson). Splenocytes were prepared using standard protocols. For the measurement of BrdU incorporation, mice were inoculated with 1 mg BrdU (Sigma) intraperitoneally 24 hr prior to sacrifice. Splenocytes were prepared and stained with both B220 and BrdU antibodies using reagents from Pharmingen-Becton Dickinson. To validate BrdU incorporation, thymocytes were also prepared from the same animals. BrdU staining was verified in a range of 18% to 22% of CD3-positive thymocytes. No difference was evident between wild-type and TACI null mice. BLyS binding was determined by incubating splenocytes with Flag-tagged human recombinant BLyS (J. Gross, Zymogenetics, Inc.) at 100 ng /10^6 cells in HBSS with 1% BSA. Following washes, cells were consecutively incubated with biotinylated anti-Flag antibodies (Sigma), phycoerythrin-conjugated streptavidin (Jackson Immunoresearch), and FITC-conjugated antibodies against B220.

Immunofluorescence

Fresh spleens were frozen in tissue freezing medium (Triangle Biomedical Sciences), and 10 μm sections were prepared with a cryostat (Leica). Sections were fixed in acetone and blocked with 1% gelatin and 1% normal rat serum in PBS. Antibody and lectin incubations were in PBS with 0.1% gelatin as indicated on the figure legends. Sections were washed with PBS between incubations, mounted with Vectashield (Vector Laboratories), and visualized with a confocal microscope (Zeiss LSM 510).

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