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B cell ADAM17 Controls T cell Independent Humoral Immune Responses through Regulation of TACI and CD138

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Abstract

ADAM17 is known to contribute to the immune system through its shedding of tumor necrosis factor alpha (TNF α). However, the role of ADAM17 in B cell biology is not well characterized. To determine whether B cell ADAM17 contributes to T cell-independent humoral immune responses, we crossed CD19 Cre transgenic mice with mice bearing a floxed allele of ADAM17 (ADAM17^{CD19}). In this study, we show a B cell intrinsic role for ADAM17 in regulating marginal zone B cell (MZB) numbers in mice. Interestingly, we demonstrate that the loss of B cell ADAM17 results in reduced MZB numbers in the naïve state and after immunization with T-independent antigen, yet enhanced humoral immunity to T cell independent antigens. We additionally find elevated TACI and CD138 levels on plasma cells following immunization in ADAM17^{CD19} mice. Overall, these findings suggest that B cell ADAM17 may orchestrate T independent immune responses through both MZB numbers and plasma cell antibody production.

Keywords

ADAM17; Plasma cell; Marginal zone B cell; humoral immunity; T cell independent

Introduction

ADAM17 in the immune system has been well characterized, however, studies examining the role of ADAM17 in B cell biology have been limited [1]. Classically, ADAM17 was shown to contribute to inflammation due to its ability to shed TNF α , ultimately leading to its early name TNF α converting enzyme (TACE) [1]. Since this discovery, numerous immunologically relevant molecules including TNF receptors, IL6R α , as well as many other cytokine and chemokine receptors have been added as substrates of ADAM17 [1].

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Studies on B cell ADAM17 suggested a potential role for ADAM17 in the regulation of ICOSL. This was later shown by our laboratory to primarily be the case with non-physiological stimuli such as PMA or in the absence of ADAM10, ICOSL's primary sheddase [2,3]. Additionally, it was shown that loss of B cell ADAM17 has a limited role on T cell dependent (TD) immunizations, with only a small increase in IgG2a[2] and no effect on follicular helper T cell development [3]. However, the role of ADAM17 in T cell independent (TI) immunizations and marginal zone B cell (MZB) function is not known.

MZBs are found in the marginal zone of the spleen, which is located between the white pulp and the red pulp separated by the porous, vascular marginal sinus. Most of the blood that circulates into the spleen enters through the marginal sinus where it can then enter the marginal zone into the red pulp and back through the venous sinus [4–6]. The location of MZBs in the marginal zone makes them unique. Continuously exposed to blood, MZBs rapidly sense blood-borne pathogens. MZBs are necessary for responses to encapsulated bacteria and TI-2 antigens [7–10]. Mice lacking MZBs due to defects in development, such as loss of Pyk-2, ADAM10, or RBP-J κ also have defects in responses to TI-2 antigens [11–14].

Activated MZBs rapidly differentiate into plasmablasts following immunization with TI-2 antigens [15]. The ability of MZBs to differentiate into plasmablasts relies heavily on CD11c^{lo} DCs that take up TI-2 antigens in circulation [16]. In addition to bringing antigen to the marginal zone, these DCs secrete B cell activating factor (BAFF) and A proliferation inducing ligand (APRIL) which signal through transmembrane activator and calcium-modulating/cyclophilin ligand-interacting protein (TACI), B-cell maturation antigen (BCMA), and BAFF-R to provide survival signals and a co-signals required for plasmablast generation [16]. TACI is constitutively shed by ADAM10, which releases a soluble TACI (sTACI) that can act as a decoy receptor [17]. It is also shed by ADAM17, this shedding is regulated by the aggregation of BAFF. BAFF 3-mers can cause the shedding of BAFF-R by ADAM10 but does not cause shedding of TACI while BAFF 60-mers are able to cause TACI and BAFF-R shedding by both ADAM10 and ADAM17 [18]. Different B cell subsets rely on ADAM10 or ADAM17 for the regulation of TACI and BAFF-R.

Heparan sulfate proteoglycans (HSPGs) are proteins with heparan sulfate chains and are expressed in all mammalian tissue as extra-cellular matrix components as cell membrane bound proteins [19]. Plasma cells upregulate HSPG syndecan-1 (CD138). Original studies demonstrated that CD138 deficient mice develop normally and are able to produce antigen-specific antibody responses [20]. These findings suggest that CD138 is not necessary for plasma cell function, but these studies did not thoroughly examine plasma cell numbers. More recent studies examining CD138 deficient mice showed that following TD immunization with NP-KLH, exhibited decreased antigen specific IgG titers at day 7, 28, and 60 [21]. This group additionally showed that loss of CD138 caused increased apoptosis in plasma cells due to decreased APRIL and IL-6 signaling [21].

Here, we demonstrate a multifactorial role for ADAM17 in humoral immune responses to TI immunizations. Using mice with ADAM17 deleted in B cells (ADAM17^{Cd19}), we show that loss of B cell ADAM17 results in a decreased MZB numbers while increasing humoral

responses to TI-2 immunizations. These findings shed new light on the role of ADAM17 in both TACI and CD138 regulation in MZBs as well as plasma cells.

Materials and Methods

Mice

Mice were maintained at the Virginia Commonwealth University Animal Facility in accordance with guidelines by the U.S. National Institutes of Health and American Association for the Accreditation of Laboratory Animals Care. Mice with Exon 2 of *Adam17* floxed were (The Jackson Laboratory; 009597) bred to *Cd19*-Cre knockin mice (The Jackson Laboratory; 006785) to create ADAM17^{Cd19} mice. Mice were genotyped using high resolution melting (HRM) assays. DNA was isolated as in [3]. Primers for Cre and Adam17 flox site have been previously published [3,22].

Microscopy

Spleens were frozen on dry ice in OCT compound (Tissue-Tek). 10µm sections were cut from frozen blocks using a cryostat (Frigocut 2800E; Jung), fixed in absolute ice-cold acetone for 10 minutes and air-dried. Sections were rehydrated in PBS and blocked in 4% horse serum and 3% bovine serum albumin (BSA) in PBS for 60 minutes at room temperature. Sections were washed and labelled with antibodies at indicated concentrations (Supplemental Table 1) for 60 minutes at room temperature. Images were captured on a LSM700 Axio Imager 2 (Zeiss).

Flow Cytometry

Single cell suspensions of all organs were stained for live-dead exclusion using Zombie Aqua (Biolegend, 423102) according to manufacturer's protocol. Cells were washed with FACS buffer (5% fetal bovine serum (FBS) in phosphate buffered saline (PBS) with 2mM EDTA). Fc receptors were blocked with 5µg 2.4G2 (130) for 10 minutes at 4°C. Antibodies were added at indicated concentrations (Supplemental Table 1) for 45 minutes at 4°C. Cells were fixed in Fixation Buffer (BioLegend, 420801) for 10 minutes at room temperature. Flow cytometry data was collected on an LSR Fortessa X-20 (BD) and analyzed in FlowJo version 10 (BD).

Immunizations

20µg of 4-hydroxy-3-nitrophenylacetatyl hapten conjugated to Ficoll (NP₄₀-Ficoll; BioSearch Technologies) or 100µg of 4-hydroxy-3-nitrophenylacetatyl hapten conjugated to keyhole limpet hemocyanin (NP₃₅-KLH; BioSearch Technologies) was dissolved in 100µL of sterile saline and injected *i.p.*

Antibody Quantification

Blood was collected by tail vein bleeding or cardiac puncture. Serum was then separated by centrifugation and stored at -20°C until use. Total and NP-specific antibody quantification was as previously described [23].

Statistics

Statistical analyses and graphing were conducted using Graphpad Prism 8.0. All data was analyzed with a student's T test when comparing two groups and one-way ANOVA with Tukey's post-test when more than 2 groups. Experiments shown were repeated at least twice independently. If p values are not indicated, the data is not statistically significant.

Results

B cell ADAM17 loss alters MZB development

To examine the role of ADAM17 in B cells, we looked at naïve ADAM17^{Cd19} mice for total B cell numbers in the spleen. There was no difference in relative or absolute total B cell numbers between ADAM17^{Cd19} and WT mice (Fig. 1A). Additionally, there was no difference in the relative or absolute follicular B cell (FoB) numbers between WT and ADAM17^{Cd19} mice (Fig. 1B). However, we did observe a significant decrease in the relative and absolute number of MZBs in the spleen of ADAM17^{Cd19} mice compared to WT (Figs. 1C–E). This decrease was also observed using an immunofluorescence assay (IFA; Fig. 1F). Additionally, we did not see any difference in the relative number of transitional B cell subsets between WT and ADAM17^{Cd19} mice (Fig. 1G).

With the similarity in the innate immune sensing properties of MZBs and B1 cells, we next wanted to examine the B1 cell compartment in ADAM17^{Cd19} mice (Fig. 1H). ADAM17^{Cd19} mice had a significantly lower relative number of B1 cells compared to WT (Fig. 1H). Both B1a and B1b subsets of B1 cells were significantly decreased (Fig. 1H). However, we did not see a difference in the relative number of peritoneal cavity B2 cells in ADAM17^{Cd19} mice compared to WT (Fig. 1H).

B cell ADAM17 loss does not alter TD humoral immune responses

We next examined whether there was any effect of B cell ADAM17 loss in TD humoral immune responses. To do this, we immunized WT and ADAM17^{Cd19} mice with NP₃₅-KLH in alum *i.p.* and boosted the mice 21 days later. Antibody levels were measured 7 days post boost from serum by ELISA. There was no difference in total IgM, IgG1, IgG2a or IgG3 between WT and ADAM17^{Cd19} mice (Fig. 2A). We additionally observed no difference in antigen-specific, NP₂₅-BSA-binding (total affinity) or NP₄-BSA-binding (high affinity) IgM, IgG1, IgG2a, or IgG3 (Figs. 2B, C).

Loss of B cell ADAM17 enhances MZB responses to TI-2 immunizations

As MZBs are essential for responses to TI-2 immunizations, we next examined whether ADAM17^{Cd19} mice had any defects in TI-2 humoral responses. We immunized ADAM17^{Cd19} and WT mice with 20µg of NP-Ficoll *i.p.* and analyzed the mice for humoral responses. ADAM17^{Cd19} mice had significantly lower relative and absolute MZB numbers compared to WT mice at day 7 post-immunization (Fig. 3A,B).

At day 0, total IgM levels were not different but were significantly elevated at day 7 in ADAM17^{Cd19} mice compared to WT (Fig. 3C). Interestingly, total IgG3 levels were significantly elevated at day 0 in ADAM17^{Cd19} mice compared to WT mice as well as day 7

post-immunization (Fig. 3D). ADAM17^{Cd19} mice had significantly higher levels of total affinity IgM (Fig. 3E), IgG_{2a} (Fig. 3F), and IgG₃ (Fig. 3G) compared to WT mice 7 days post-immunization. Additionally, when we measured high affinity responses 7 days post-immunization, we saw significantly increased levels of high affinity IgM in ADAM17^{Cd19} mice compared to WT (Fig. 3H). While we saw a trend towards increased high affinity IgG₃ in ADAM17^{Cd19} mice, this trend was not significant (Fig. 3I). In concordance with our antibody data, we saw significantly increased numbers of NP-PE⁺ MZBs in ADAM17^{Cd19} mice by flow cytometry when compared to WT mice at day 7 post-immunization (Fig. 3J–K).

ADAM17 Regulates Plasma Cell Survival Markers

With our findings of increased antibody antigen specific antibody responses to NP-Ficoll immunization, even in the presence of decreased MZB numbers, we next wanted to examine plasma cells. To do this, we examined the spleens of NP-Ficoll immunized mice 7 days post immunization. We saw no significant difference in relative or absolute splenic plasma cell numbers between WT and ADAM17^{Cd19} mice (Figs. 4A–B).

As TACI is important for MZB and plasma cell function, we examined the levels of this protein on MZBs and plasma cells from naive and NP-Ficoll immunized mice. MZBs from ADAM17^{Cd19} mice were not significantly different in the level of TACI on the cell surface (Figs. 4C–D). However, when we examined plasma cells, we saw a significant increase in TACI levels in ADAM17^{Cd19} mice compared to WT mice at day 7 post-immunization (Fig. 4E–F).

Additionally, as CD138 (Syndecan-1) has a unique ability to bind survival factors such as APRIL, which can also help to increase TACI signaling and has been shown to play a role in antibody production, we next sought to examine whether CD138 levels were affected in ADAM17^{Cd19} mice. When examining plasma cells from ADAM17^{Cd19} mice compared to WT mice, we observed significantly higher levels of CD138 on plasma cells from ADAM17^{Cd19} mice compared to WT plasma cells 7 days post NP- Ficoll immunization (Fig. 4G–H).

Discussion

There have been several findings that prompted us to examine the role of ADAM17 in B cell function. It was previously reported that ADAM17 was involved in ICOSL shedding from B cells in response to PMA [2]. Since then, we have confirmed that ADAM17 is able to shed ICOSL in response to PMA but is not responsible for physiological or ICOS-mediated ICOSL shedding on B cells [3]. Additionally, *Marczynska et al.* observed increased lymph node size following TD immunization models in ADAM17 hypomorphic mice [2]. During these TD immunization models, they also observed increased levels of antibody titers in these ADAM17 hypomorphic mice, particularly IgG_{2a}. However, in our model using ADAM17^{Cd19} mice, we did not see any differences in humoral responses to TD immunizations. However, it should be noted that our adjuvant conditions were slightly different as well as the use of B cell specific ADAM17 knockout mice rather than ADAM17 hypomorphic mice.

While we saw no defect in TD responses (Fig. 2A–C), we did see significant impairment of the MZB compartment in ADAM17^{Cd19} mice (Fig. 1C–F). We did not see a difference in the number of T1 or T2 B cells in the spleens of ADAM17^{Cd19} mice compared to WT mice (Fig. 1G). This is consistent with other models in which MZB cells are absent, such as ADAM10 or Taok3 B cell knockouts where both T1 and T2 B cell compartments were intact [12,24]. This suggests that MZB survival in the absence of ADAM17 may be decreased, although confirmation of this mechanism will need further investigation.

Upon NP-Ficoll immunization, we observed significantly fewer MZB numbers in ADAM17^{Cd19} mice compared to WT mice (Fig. 3A,B). With the decrease in MZB numbers in ADAM17^{Cd19} mice in both a naïve and NP-Ficoll immunized state, we expected that these mice would also have a decreased level of antibody, especially antigen specific antibody following NP-Ficoll. However, ADAM17^{Cd19} mice had increased total affinity IgM, IgG_{2a} and IgG₃ and high affinity IgM 7 days post-immunization, suggesting enhanced somatic hypermutation and increased antibody production on a per cell basis (Fig. 3 C–G).

With the finding of decreased MZB numbers and increased antigen specific antibody responses in ADAM17^{Cd19} mice, we next wanted to examine whether this could be attributed to altered TACI levels on MZBs. When we examined MZBs for TACI levels following NP-Ficoll immunization, we saw no difference in TACI levels between ADAM17^{Cd19} mice and WT, (Fig. 4C,D). However, in addition to being expressed on MZBs, TACI is highly expressed on plasma cells. While we did not see a difference in absolute plasma cell numbers between ADAM17^{Cd19} and WT mice following NP-Ficoll immunization, plasma cell TACI levels were significantly increased in ADAM17^{Cd19} mice compared to WT mice (Fig. 4 E,F).

The role of TACI in B cell biology is complex and not fully understood. The earliest studies examining TACI in B cell biology found that global loss of TACI resulted in increased MZB cell numbers, yet drastically decreased antigen specific antibody responses following TI-2 immunization models [25,26]. These findings could be explained by defective signaling to B cells, or may be explained with the recent findings that soluble TACI can act as a decoy receptor, decreasing the overall pool of BAFF and APRIL available for B cells [17]. However, mice that overexpress APRIL actually have enhanced antigen specific and class switched antibody responses to TI-2 immunization models [27,28]. These findings also support the notion that sTACI may act as a decoy receptor but also show the importance of APRIL-mediated TACI stimulation in proper TI-2 immunizations. However, studies using APRIL overexpression were not able to deduce whether the increased antigen-specific TI-2 responses were due to increased MZB responses or increased antibody production by plasma cells, since both rely on APRIL [27]. However, TD responses in APRIL overexpressing mice have increased IgM but not IgG titers compared to WT mice, suggesting that APRIL overexpression affects MZBs more drastically than plasma cells [28].

In ADAM17^{Cd19} mice, we hypothesize that the increased TACI levels on plasma cells is due to decreased proteolytic shedding as a result of ADAM17 loss. TACI has been shown to be cleaved by both ADAM10 and ADAM17 in a seemingly cell specific context [18]. Neither murine MZBs nor plasma cells have been examined previously to determine whether

ADAM10 or ADAM17 cleaves TACI in these subsets. We therefore hypothesize that the increased levels of TACI on plasma cells would lead to increased antibody production in ADAM17^{Cd19} mice. In addition to elevated TACI levels on plasma cells in ADAM17^{Cd19} mice, we saw significantly increased levels of CD138 (Fig. 4 G,H). With CD138's ability to bind APRIL and increase its multimerization and signaling potential, we hypothesize that in addition to increased APRIL-mediated signaling in plasma cells due to increased TACI levels, increased CD138 levels act synergistically in this signaling pathway.

Taken together, these findings of increased antibody responses in a TI-2 immunization model in ADAM17^{Cd19} mice as well as altered surface receptors is an important step in our understanding of both MZB biology as well as ADAM17 biology. These studies further refine our understanding of ADAM17 in MZB biology and TI-2 humoral immune responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Loss of B cell ADAM17 results in decreased marginal zone B cell accumulation
- B cell ADAM17 regulates humoral responses to T-independent but not T-dependent antigens.
- ADAM17 regulates plasma cell CD138 and TACI levels

Figure 1: ADAM17^{Cd19} mice have a defect in the marginal zone compartment.

(A) Quantification of the absolute number of CD19⁺ B220⁺ B cells in the spleens of naïve mice. (B) Quantification of the absolute number of FoBs in the spleens of naïve mice. (C) Representative flow cytometry plot of CD19⁺ B220⁺ B cells from naïve WT and ADAM17^{Cd19} mice gating for MZBs. (D) Quantification of the relative percent of MZBs as a percent of total B cells. (E) Quantification of the absolute number of MZBs from the spleens of naïve mice. (F) Immunofluorescence assay of spleens from naïve WT and ADAM17^{Cd19} mice showing marginal zones. Staining shows CD169 (green), IgD (red) and IgM (blue). (G) Relative quantification of transitional B cell populations in the spleens of naïve WT and ADAM17^{Cd19} mice. (H) Relative quantification of B cell populations from the peritoneal cavity of naïve WT and ADAM17^{Cd19} mice. Statistical analysis by student's T-test (A, B, D, E) and one-way ANOVA with Tukey's post-test (G, H).

*p<0.05; ****p<0.0001. Data is representative of 2 independent repeats.

Figure 2: ADAM17^{Cd19} mice have normal TD responses.

(A) Total antibody levels from WT and ADAM17^{Cd19} mice 7 days post NP-KLH boost. (B) Total affinity (NP-25 binding) antibody levels from WT and ADAM17^{Cd19} mice 7 days post NP-KLH boost. (C) High affinity (NP-4 binding) antibody levels from WT and ADAM17^{Cd19} mice 7 days post NP-KLH boost. Statistical analysis: one-way ANOVA with Tukey's post-test (A - C). Representative of 2 independent repeats.

Figure 3: ADAM17^{Cd19} mice have enhanced responses to TI-2 immunizations.

(A) Representative flow cytometry plots of WT and ADAM17^{Cd19} mice 7 days post-immunization with NP-Ficoll with quantification of relative numbers of MZBs. (B) Quantification of the absolute number of MZBs 7 days post-immunization with NP-Ficoll. Total IgM (C) and IgG3 (D) 7 days post-immunization with NP-Ficoll as measured by ELISA. Total affinity (NP-25 binding) IgM (E), IgG2a (F), and IgG3 (G) 7 days post-immunization with NP-Ficoll as measured by ELISA. High affinity (NP-4 binding) IgM (H) and IgG3 (I) 7 days post-immunization with NP-Ficoll as measured by ELISA. (J) Representative flow cytometry plots of MZBs from WT and ADAM17^{Cd19} mice 7 days post-immunization with NP-Ficoll that are NP binding. (K) Statistical analysis: student's T-test (B, H, I, K), one-way ANOVA with Tukey's post-test (C - G).

*p<0.05; **p<0.001; ***p<0.0001. Representative of 2 independent repeats.

Figure 4: ADAM17 regulates TACI and CD138 on plasma cells.

(A) Representative flow cytometry plots of CD45⁺ cells from WT and ADAM17^{Cd19} mice 7 days post-immunization with NP-Ficoll gating for CD138⁺ cells in the spleen. (B) Quantification of A. (C) Histogram of the MFI of TACI expression on MZBs 7 days post-immunization with NP-Ficoll as measured by flow cytometry. (D) Quantification of C. (E) Histogram of the MFI of TACI expression on CD138⁺ cells 7 days post-immunization with NP-Ficoll as measured by flow cytometry. (F) Quantification of E. (G) Histogram of the MFI of CD138 expression on CD138⁺ cells 7 days post-immunization with NP-Ficoll as measured by flow cytometry. (H) Quantification of G. Statistical analysis: student's T-test (B, D, F, H). ***p<0.0001. Representative of 2 independent repeats.