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A Disintegrin and Metalloproteinase 10 Regulates Antibody Production and Maintenance of Lymphoid Architecture

Natalia S. Chaimowitz,*‡ Rebecca K. Martin,*‡ Joanna Cichy,† David R. Gibb,* Pooja Patil,* Dae-Joong Kang,* Julie Farnsworth,‡ Eugene C. Butcher,§ Brent McCright,¶ and Daniel H. Conrad*

A disintegrin and metalloproteinase 10 (ADAM10) is a zinc-dependent protease related to matrix metalloproteinases. ADAM10 has emerged as a key regulator of cellular processes by cleaving and shedding extracellular domains of multiple transmembrane receptors and ligands. We have developed B cell-specific ADAM10-deficient mice (ADAM10<sup>B<sup>−/−</sup></sup>). In this study, we show that ADAM10 levels are significantly enhanced on germinal center B cells. Moreover, ADAM10<sup>B<sup>−/−</sup></sup> mice had severely diminished primary and secondary responses after T-dependent immunization. ADAM10<sup>B<sup>−/−</sup></sup> displayed impaired germinal center formation, had fewer follicular Th cells, decreased follicular dendritic cell networks, and altered chemokine expression in draining lymph nodes (LN). Interestingly, when spleen and LN structures from immunized mice were analyzed for B and T cell localization, tissues structure was aberrant in ADAM10<sup>B<sup>−/−</sup></sup> mice. Importantly, when ADAM10-deficient B cells were stimulated in vitro, they produced comparable Ab as wild type B cells. This result demonstrates that the defects in humoral responses in vivo result from inadequate B cell activation, likely because of the decrease in follicular Th cells and the changes in structure. Thus, ADAM10 is essential for the maintenance of lymphoid structure after Ag challenge. The Journal of Immunology, 2011, 187: 000–000.
CD23 (15, 16). Moreover, ADAM10 has been identified as a key regulator of lymphocyte development. We and others have demonstrated that ADAM10 is critical for T cell and marginal zone B cell (MZB) development (17, 18). However, the role of ADAM10 in mature B cell function remains unknown and warrants further studies.

In this study, we show that Peyer’s patches GCs have elevated ADAM10 expression. Recently, we generated mice with a B cell-specific deletion of ADAM10 (ADAM10<sup>B<sup>-/-</sup></sup>) (18) and showed that MZBs were missing because of defective Notch2 cleavage. In this article, we demonstrate that ADAM10<sup>B<sup>-/-</sup></sup> mice have impaired GC formation and markedly diminished Ab responses to T-dependent Ags. Overexpression of the Notch2 intracellular domain (N2ICD) did not correct the defect. Interestingly, ADAM10-deficient B cells can class switch normally when stimulated in vitro, suggesting that the phenotype observed in vivo results from insufficient B cell activation, likely because of decreased T cell help. Consistent with this hypothesis, T<sub>B<sub>0 numbers were also diminished. Furthermore, clear B and T cell segregation was observed in secondary lymphoid tissues prior to challenge. The defective humoral response seen in ADAM10<sup>B<sup>-/-</sup></sup> mice was associated with altered chemokine expression, loss of FDC networks, and changes in T and B cell localization within secondary lymphoid organs. These results demonstrate that B cell-expressed ADAM10 is critical for the maintenance of lymphoid architecture and proper positioning of T and B cells during immune responses.

Materials and Methods

Mice and immunizations

ADAM10<sup>B<sup>-/-</sup></sup> mice were previously described (18). To generate ADAM10<sup>B<sup>-/-</sup>×N2ICD-Tg<sup>+</sup></sup> mice, we bred ADAM10<sup>B<sup>-/-</sup></sup> mice with N2ICD-Tg<sup>+</sup> mice (19). CD19<sup>cre</sup> mice were wild type (WT) littermates of ADAM10<sup>B<sup>-/-</sup></sup> and ADAM10<sup>B<sup>-/-</sup></sup>×N2ICD-Tg<sup>+</sup> mice. CD23Tg mice have been previously described. BALB/cJ mice were used as littermate controls of CD23Tg mice (20). All mouse protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

In vitro activation

Splenocytes were cultured (12.5 × 10<sup>6</sup> cells/well) with CD40L-transfected Chinese hamster ovary cells as a B cell stimulant (6 × 10<sup>5</sup> cells/well) and IL-4 (10,000 units/ml) for 7 d. Supernatants were then harvested and analyzed for IgG1 levels by ELISA.

Flow cytometry

Cell isolation and labeling was conducted as described previously (18, 21). Cells were labeled after RBC lysis and filtration through 40-μm cell strainers. Abs included anti-mouse unlabeled CD16/32 (2.4G2), biotinylated CXCR5 (RPB32); PE-conjugated B220 (RA3-6B2), IgD (11C2), CD21/CD35 (7E9); FITC-conjugated Thy1.2 (53-2.1); CD21/CD35 (7E9) and CD3 (17A2) from BioLegend; FITC-conjugated GL7 from eBioscience. Sections were washed, mounted with antifade mounting medium (Vectashield; Vector Laboratories), coverslipped, and examined with a confocal laser-scanning microscope (TCS-SP2 AOBS; Leica) fitted with an oil Plan-Apochromat 40× objective. Two lasers were used: argon for FITC and PE and He/Ne for allophycocyanin. Parameters were adjusted to scan at ≤ 512 × 512 pixel density for Fig. 1 and ≤ 1024 × 1024 pixel density for the confocal images. Emissions were recorded in separate channels. Digital images were captured, overlaid, and processed with the Confocal and LCS Lite programs (Leica).

Statistical analysis

The p values were calculated using unpaired two-tailed Student t tests in GraphPad Prism. Error bars represent the SEM between samples. A p value ≤ 0.05 is considered significant.

Results

ADAM10 is highly expressed in GC B cells

Delineation of ADAM10 expression patterns in B cells is an important step toward understanding the potential role of the proteinase in humoral immunity. Flow cytometry analysis demonstrated that, within Peyer’s patches, although ≤ 2% of naive B cells expressed ADAM10, > 80% of GCB cells were ADAM10<sup>+</sup> (Fig. 1A, 1B). The flow cytometric results were confirmed by immunohistochemistry of Peyer’s patches stained for ADAM10, peanut agglutinin (PNA), and IgD. Consistent with our flow cytometry results, ADAM10 was highly expressed by cells within GCS (Fig. 1C). Peyer’s patches were chosen because of the expected high level of GC activation; however, in other experiments (data not shown), elevated levels of ADAM10 were also seen on B cells from the draining LNs of immunized mice.

Humoral immune responses in ADAM10<sup>B<sup>-/-</sup></sup>-deficient mice

Given the significant expression of ADAM10 in GC B cells, we investigated its role in humoral responses by using B cell-specific

ADAM10 as a real-time PCR machine (iQ5; Bio-Rad Laboratories). Primers and probes for running a TaqMan quantitative PCR assay were purchased from Applied Biosystems. TaqMan gene expression assays included CCL19: Mm00839967_g1 and IL-21: Mm00517640_m1. Primers for CCL21 were synthesized by IDT as previously described (22). PCR products, labeled with 6-FAM-conjugated probes, were amplified with 18S as an internal control. Reaction parameters were as follows: hold at 48°C for 30 min and hold at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Results were analyzed with iQ5 real-time PCR software (version 2.0).

ELISA and ELISPOT

For ELISA, serum was collected from mice at different time points after immunization. Samples were diluted serially and added to 96-well plates (50 μl/well) precoated with NP-14–BSA (15 μg/ml; Biosearch Technologies). After incubation at 37°C for 1 h, biotinylated anti-mouse Ig (Southern Biotech) was added to plates. After a second incubation at 37°C for 1 h, bound Abs were revealed by Streptavidin-AP (Southern Biotech). For measurement of high-affinity Abs, protocol was carried out as described except that plates were coated with NP-4–BSA (Biosearch Technologies; 15 μg/ml). Standard curves were performed by coating with anti-IgM or anti-IgG and adding known amounts of IgM or IgG, respectively. The values for experimental samples are reflected as relative units because serum Ag-specific Abs were captured with Ag. For ELISPOT, 96-well MultiScreen filtration plates (Millipore) were coated with NP-4–BSA or NP-14–BSA. Although only high-affinity Abs can efficiently bind NP-4–BSA, and both low- and high-affinity Abs bind NP-14–BSA, this ELISA system allows for the determination of high- versus low-affinity Abs (23). Total splenic cells were seeded into the plate in duplicate and cultured for 18 h at 37°C. Plates were developed as previously described (24).

Immunofluorescence and confocal microscopy

Spleens were frozen on dry ice in OCT compound (Tissue-Tek; Sakura). Serial 10-μm sections were cut from frozen blocks using a cryostat (Frigocut 2800E; Jung), fixed in absolute acetone, and air-dried. Sections were blocked with 10% BSA in PBS to prevent background staining and then washed and incubated for 60 min with different combinations of 2–5 μg/ml anti-mouse Abs. Abs included conjugated B220 (RA3-6B2) and Igs (11-26c.2a), FITC-conjugated Thy1.2 (53-2.1), CD21/CD35 (7E9) and CD3 (17A2) from BioLegend; FITC-conjugated GL7 from eBioscience. Sections were washed, mounted with antifade mounting medium (Vectorshield; Vector Laboratories), coveredslipped, and examined with a confocal laser-scanning microscope (TCS-SP2 AOBS; Leica) fitted with an oil Plan-Apochromat 40× objective. Two lasers were used: argon for FITC and PE and He/Ne for allophycocyanin. Parameters were adjusted to scan at ≤ 512 × 512 pixel density for Fig. 1 and ≤ 1024 × 1024 pixel density for the confocal images. Emissions were recorded in separate channels. Digital images were captured, overlaid, and processed with the Confocal and LCS Lite programs (Leica).

Statistical analysis

The p values were calculated using unpaired two-tailed Student t tests in GraphPad Prism. Error bars represent the SEM between samples. A p value ≤ 0.05 is considered significant.
ADAM10-deficient mice (ADAM10<sup>B</sup>-/−). Basal levels of serum IgM, IgG1, IgG2a, and IgG2b were significantly reduced in ADAM10<sup>B</sup>-/− mice when compared with littermate or heterozygous (not shown) controls, suggesting a defect in Ab production (Fig. 2A). Because cre expression reduces the CD19 expression by about half, this result also shows that the decrease in Ab responses is not secondary to decreased CD19 expression. To directly assess Ag-specific Ab production, we immunized ADAM10<sup>B</sup>-/− mice i.p. with NP-KLH and examined serum levels of NP-specific Ab. ADAM10<sup>B</sup>-/− mice showed reduced levels of NP-specific IgM 7 d postimmunization; however, levels reached control values by day 14 and through the remainder of the experiment (Fig. 2B). In contrast, NP-specific IgG levels, both total and high affinity, were reduced 4 wk postimmunization (Fig. 2B). This reduction in IgG was not specific to a particular isotype, as Ag-specific IgG1, IgG2a, and IgG2b were dramatically reduced in ADAM10<sup>B</sup>-/− mice (Supplemental Fig. 1A). Similar results were obtained when mice were immunized s.c. (results not shown). Furthermore, even when immunization with a high Ag dose, 1 mg NP-KLH, was performed, ADAM10<sup>B</sup>-/− mice failed to mount a normal Ab response, demonstrating that the defect in Ab production cannot be overcome by simply providing more Ag (Supplemental Fig. 1B).

Littermate controls and ADAM10<sup>B</sup>-/− mice were immunized and boosted 42 d later to test whether memory responses were also impaired in ADAM10<sup>B</sup>-/− mice. Consistent with data shown in Fig. 2B, ADAM10<sup>B</sup>-/− mice had decreased levels of Ag-specific Abs after primary immunization. Whereas littermate controls exhibited a 12-fold increase in anti-NP IgG Abs 5 d after secondary immunization (from 38.34 ± 25 to 461.5 ± 72.45), ADAM10<sup>B</sup>-/− mice had a 4-fold increase (from 3.133 ± 2.888 to 12.36 ± 6.4460). Five days after boost, control mice produced 16 times more high-affinity Ag-specific IgG and 37 times more total NP-specific IgG than ADAM10<sup>B</sup>-/− mice (Fig. 2C). These data demonstrate that in ADAM10<sup>B</sup>-/− mice, B cell memory responses are impaired.

**PC development is impaired in ADAM10<sup>B</sup>-/− mice**

Examination of PC generation by ELISPOT assays showed that ADAM10<sup>B</sup>-/− mice that had been immunized had drastically reduced numbers of NP-specific Ab-secreting cells (ASCs) in spleen and bone marrow 21 d after immunization (Fig. 3A, 3B). Similar results were obtained at 14 and 28 d after immunization (results not shown). These results demonstrate that the decrease in splenic ASCs is not due to altered migration or preferential localization to the bone marrow, but likely due to impairments in PC differentiation, survival, or both.

A critical component of the humoral response is affinity maturation. Thus, we next examined affinity maturation in immunized ADAM10<sup>B</sup>-/− mice. Whereas 21 d after immunization most ASCs were secreting high-affinity Abs in littermate controls, the few ASCs that were present in ADAM10<sup>B</sup>-/− mice were secreting low-affinity Ab. Littermate controls had 12-fold higher number of NP-specific ASCs and 20-fold higher number of NP-specific ASCs producing high-affinity Abs when compared with ADAM10<sup>B</sup>-/− mice. These results suggest a defect in affinity maturation in ADAM10<sup>B</sup>-/− mice (Fig. 3C).

**GC formation is impaired in ADAM10<sup>B</sup>-/− mice**

Fig. 3 demonstrated that Ag-specific ASCs were reduced in immunized ADAM10<sup>B</sup>-/−. That long-lived PCs and memory cells are generated within GCs (9), GC formation in ADAM10<sup>B</sup>-/− mice was examined. GC B cells, defined as IgM<sup>B</sup>IgD<sup>B</sup>B220<sup>E</sup> IgG1<sup>C</sup>CD38<sup>B</sup>, were enumerated by flow cytometry (25) (Fig. 4A). Remarkably, the number and percentage of GC B cells within the mucosal epithelium (E) also reacts with PNA and anti-ADAM10.

**FIGURE 1.** ADAM10 expression on GC B cells. **A** and **B**, Expression of ADAM10 on gated GC PNA<sup>A</sup>IgD<sup>D</sup> (blue) and naive PNA<sup>B</sup>IgD<sup>B</sup> (red) B (CD19<sup>E</sup>) cells isolated from Peyer’s patches. Flow cytometry gates used to define the GC and naive B cell populations are indicated on the left. To demonstrate specificity of staining, we used isotype-matched mAb instead of mAb against ADAM10 (**left panel**). Original magnification ×20. The mucosal epithelium (E) also reacts with PNA and anti-ADAM10. **C**, Frozen serial sections of Peyer’s patches were stained to detect B cell follicles (B220<sup>E</sup>, blue), GCs (PNA<sup>A</sup>, green), and ADAM10 (red) (**left panel**). To demonstrate specificity of staining, we used isotype-matched mAb instead of mAb against ADAM10 (**right panel**).
spleen was dramatically decreased in ADAM10B<sup>−/−</sup> mice (Fig. 4B, 4C). Similar results were obtained when draining LNs were analyzed (data not shown).

**ADAM10B<sup>−/−</sup> mice have altered splenic and LN architecture, as well as dysregulated chemokine expression after Ag challenge**

To better understand the relationship between decreased GC B cells and the defects in Ab production depicted in Fig. 2, we assessed GC formation in immunized mice by immunohistochemistry. As depicted in Fig. 5, although several GCs could be detected in littermate controls, as determined by GL7<sup>+</sup> cells, draining LNs of ADAM10B<sup>−/−</sup> mice contained a paucity of GL7<sup>+</sup> clusters. Interestingly, GL7<sup>+</sup> cells were scattered throughout the LNs of ADAM10B<sup>−/−</sup> mice (Fig. 5A). Given the unusual pattern of GL7 expression, we then analyzed B and T cell localization within the LNs. Surprisingly, the distribution of B and T cells was aberrant, suggesting that B cell-specific ADAM10 deletion leads to changes

**FIGURE 2.** ADAM10B<sup>−/−</sup> mice have impaired humoral responses. A, Serum IgM, IgG1, IgG2a, and IgG2b were measured by capture ELISA from nonmanipulated 8- to 12-wk-old mice. B, ADAM10B<sup>−/−</sup> mice and WT littermate controls were immunized with 10 µg NP-KLH emulsified in alum. At the indicated times, serum samples were collected and total NP-specific IgM, total IgG, and high-affinity IgG Ab titers were determined by ELISA, with NP-16–BSA as capture Ag for total and NP-4–BSA as capture Ag for high-affinity ELISA. C, Mice were immunized with NP-KLH emulsified in alum, rested for 42 d, and boosted with 10 µg NP-KLH for 5 d. Mice were bled weekly throughout the course of the experiment. Total and high-affinity Ag-specific IgG levels were measured by ELISA at each time point. The relative unit (RU) values for alum-injected mice were <0.001. Bars represent the mean ± SE of five to nine mice per group (*p < 0.05, **p < 0.01, ***p < 0.001). Data represent results obtained in at least two independent experiments.

**FIGURE 3.** ADAM10B<sup>−/−</sup> mice have decreased numbers of Ag-specific ASCs. ELISPOT analysis of cells producing (A) total (both low and high affinity), (B) high-affinity NP-specific IgG and (C) high versus total ratio present in spleen (top) and bone marrow (bottom) isolated from WT and ADAM10B<sup>−/−</sup> mice 21 d postimmunization with 10 µg NP-KLH emulsified in alum (*p < 0.05, ***p < 0.001). Bars represent the mean ± SE of five mice per group. Data represent results obtained in two independent experiments.
in LN structure (Fig. 5B). In contrast, LN structure in nondraining nodes or unimmunized mice were relatively normal with regard to B and T cell segregation (Fig. 5D and data not shown). Unlike the draining LNs, GL7 staining was not detected in spleen of ADAM10B<sup>−/−</sup> mice (Fig. 6A). However, although normal in unimmunized mice, splenic architecture was also clearly altered postimmunization (Fig. 6B, 6C).

Given that FDCs play a crucial role in GC formation and lymphoid tissue structure (5, 8, 26), we stained draining LN sections for FDC markers. Consistent with an absence in GCs and disorganized lymphoid architecture, relatively few FDC networks were evident in LNs and spleen from ADAM10B<sup>−/−</sup> mice (Fig. 5C, data not shown).

The organization of secondary lymphoid tissues is highly regulated by chemokine signaling. To determine whether the changes in structure observed were due to altered chemokine expression, we assessed draining and nondraining LN chemokine expression by quantitative PCR. As expected, nondraining LNs from control and ADAM10B<sup>−/−</sup> mice had similar CXCL13, CCL19, and CCL21 expression (Fig. 7A). In sharp contrast, draining LNs isolated from ADAM10B<sup>−/−</sup> mice exhibited increased CCL21 expression, whereas CXCL13 levels were comparable. CCL19 levels trended higher, but the increase was not statistically significant (Fig. 7B). Analysis of CCL21 expression by immunohistochemistry revealed diffuse staining in the ADAM10B<sup>−/−</sup> LNs (results not shown), consistent with the localization of T cells within draining LNs (Fig. 5B). These results demonstrate that B cell-expressed ADAM10 is important for maintenance of lymphoid architecture by regulating chemokine expression during active immune responses.

FIGURE 4. GC formation after T-dependent immunization. ADAM10B<sup>−/−</sup> and WT mice were immunized with 10 µg NP-KLH emulsified in alum. Fourteen and 21 d postimmunization, flow cytometry was carried out and the presence of GC B cells (IgM<sup>lo</sup>IgD<sup>lo</sup>B220<sup>+</sup>IgG1<sup>+</sup>CD38<sup>2</sup>) in the spleen of WT and ADAM10B<sup>−/−</sup> mice was assessed. Staining protocol is depicted (A). Both percentage (B) and total number (C) of GCs were enumerated. Bars represent the mean ± SE of eight mice per group. *p < 0.05, **p < 0.01. Data are representative of three independent experiments.

FIGURE 5. Immunohistochemistry analysis of GC formation and LN architecture after T-dependent immunization. ADAM10B<sup>−/−</sup> and WT mice were immunized with 10 µg NP-KLH emulsified in alum. Fourteen days after immunization, draining LN sections were stained for (A) GL7 (green) and IgD (red); (B) Thy1.2 (green) and B220 (red); (C) CD21/35 (green) and B220 (red). D, Nondraining LNs were also stained with CD3 (green) and B220 (red). Photomicrographs are representative of three independent experiments. Original magnification ×20.
Defect in humoral responses in ADAM10<sup>B<sup>−/−</sup></sup> mice is Notch2 and CD23 independent

Recent studies have suggested that Notch signaling might be involved in Ab production and differentiation of ASCs (27, 28). ADAM10 is critical for Notch signaling. ADAM10<sup>B<sup>−/−</sup></sup> mice that have constitutively active Notch2 signaling in B cells (ADAM10<sup>B<sup>−/−</sup></sup>N2ICD-Tg<sup>B</sup>) were immunized, and Ab production, GC formation, and LN architecture were examined to determine whether the Ab production defects were due to impaired Notch signaling. As expected, expression of N2ICD recovered MZB development in these mice, demonstrating that the transgene was indeed active (Supplemental Fig. 2A). Littermate controls, ADAM10<sup>B<sup>−/−</sup></sup> and ADAM10<sup>B<sup>−/−</sup></sup>N2ICD-Tg<sup>B</sup> mice were immunized. Consistent with previous experiments, ADAM10<sup>B<sup>−/−</sup></sup> mice had significantly reduced levels of NP-specific Abs. Importantly, introduction of N2ICD transgene failed to rescue the defect in Ab production and GC formation, and did not prevent changes in LN architecture (Supplemental Fig. 2B, 2C). These results demonstrate that increased Notch signaling cannot rescue the defects in humoral responses observed in ADAM10<sup>B<sup>−/−</sup></sup> mice.

We recently reported that ADAM10 is responsible for the cleavage of the low-affinity IgE receptor, CD23 (29), and consequently, ADAM10<sup>B<sup>−/−</sup></sup> B cells have increased CD23 expression (18). Studies have revealed that CD23 overexpression led to decreased IgG1 and IgE production (20). However, when CD23Tg mice were immunized with NP-KLH, analysis of NP-specific IgG indicated that CD23Tg mice had a normal Ab response to NP-KLH (Supplemental Fig. 3). Furthermore, it has been previously reported that CD23Tg mice form GCs postimmunization with T-dependent Ags (30). These results suggest that the humoral defect observed in ADAM10<sup>B<sup>−/−</sup></sup> mice is not secondary to CD23 over-expression.
Defective Ab production seen in ADAM10\(^{B-/}\) mice can be explained by decreased B cell help

We have demonstrated that ADAM10\(^{B-/}\) mice have impaired Ab responses to T-dependent Ags, as well as changes in splenic and LN architecture. To assess whether the decreased Ab levels result from a B cell-intrinsic defect, splenocytes were stimulated with CD40L and IL-4 in vitro. Interestingly, WT and ADAM10-null B cells made comparable amounts of IgG1 (Fig. 8A), demonstrating that when adequately stimulated, ADAM10-null B cells are able to class switch normally.

Tfh cells provide B cell help via CD40L, IL-21, and IL-4, and play important roles in GC B cell survival, affinity maturation, and terminal differentiation to PCs (31). Tfh cells are characterized by high expression of CXCR5 and programmed death-1, and IL-21 production (31). Given our in vitro findings and the changes in architecture observed in ADAM10\(^{B-/}\) mice, we hypothesized that the defects in humoral responses observed resulted from inadequate B cell stimulation and decreased T cell help. Results revealed that Tfh development and/or maintenance was impaired in ADAM10\(^{B-/}\) mice, and fewer Tfh cells were present in draining LNs of ADAM10\(^{B-/}\) mice (Fig. 8B). Consistent with decreased number of Tfh cells, IL-21 message levels were also reduced (Fig. 8C). These results demonstrate that although ADAM10-deficient B cells can produce normal levels of Ab in vitro, they are unable to class switch efficiently in vivo due to decreased T cell help.

Discussion

Members of the ADAM family regulate a wide range of functions, including cell migration, proliferation, and adhesion (32). ADAM10, in particular, has been recently shown to be critical for lymphocyte development through initiation of the canonical Notch signaling pathway (17, 18). In this article, we report that ADAM10 is essential for the maintenance of splenic and LN architecture during responses to T-dependent Ags. A decrease in Ag-specific IgG production is seen both with respect to serum levels and IgG ASCs, indicating that PC differentiation is influenced. Cells producing high-affinity Abs were particularly affected, consistent with a defect in GC reactions and markedly repressed FDC-reticula. Moreover, whereas ADAM10-deficient B cells are able to class switch normally when stimulated in vitro, ADAM10\(^{B-/}\) mice have defective generation of Tfh cells. Therefore, the defective humoral response in ADAM10\(^{B-/}\) mice results from impaired B cell activation in vivo.

There are currently two clear substrates for ADAM10 in B cells: Notch and CD23 (17, 18, 29). The role of Notch signaling in mature B cell function is controversial. Although some studies have clearly shown a role for Notch in Ab production, others fail to identify such a role (27, 33). To assess the role of Notch signaling in Ab production, we overexpressed N2ICD in a B cell-specific manner. In this study, we show that restoration of Notch2 signaling in ADAM10\(^{B-/}\) mice is not sufficient to rescue Ab production or GC formation. Consistent with our findings, in vivo studies of B cell-specific RBP-Jκ-deficient mice, a key mediator of notch signaling, revealed no defects in Ab production (33). Moreover, a recently published report demonstrated that B cell-specific Notch2-deficient mice have normal GC formation (34). Studies have shown that N2ICD and N1ICD have redundant functions. Our results, therefore, suggest that the phenotype observed in ADAM10\(^{B-/}\) mice is not dependent on Notch1 or Notch2 signaling (35). However, the role of Notch1 in B cell biology should be more thoroughly studied and these studies are in progress. In vitro studies have demonstrated that Notch signaling enhances B cell activation (27) and support GC B cell survival (28). Although Notch signaling enhances humoral responses in vitro, decreased Notch signaling does not explain the phenotype observed in ADAM10\(^{B-/}\) mice. Because Notch signaling also enhances CD21 expression, this finding also suggests that CD21 is not involved in the ADAM10\(^{B-/}\) phenotype. In addition, unlike CD21/CD35\(^{−/−}\) mice, ADAM10\(^{B-/}\) mice had impaired affinity maturation and failed to mount an Ab response comparable with controls even after immunization with large Ag dosages (i.e., 1 mg) (36).

Another B cell-expressed ADAM10 substrate is CD23 (29). We have recently demonstrated that CD23 surface expression is significantly enhanced after deletion of ADAM10 in B cells (18). Defects observed in ADAM10\(^{B-/}\) could result from CD23 overexpression. Previous studies with CD23Tg mice, however,
demonstrated that elevated CD23 levels resulted in inhibition of IgE and IgG1 production, whereas other classes were not significantly affected. Furthermore, the defect was evident only after boosting (20, 37). In contrast, in ADAM10β−/− mice, all classes of IgG were influenced, and this defect was observed after just one injection. Moreover, when immunized with NP-KLH, CD23Tg mice had Ig levels comparable with that of WT. In contrast, ADAM10β−/− mice showed diminished levels of both low- and high-affinity anti-NP Abs. These results indicate that elevated CD23 levels do not explain the phenotype of ADAM10β−/− mice. It is likely that B cells express another ADAM10 substrate that has yet to be described and/or that ADAM10-mediated trans-cleavage is responsible (14).

In lymphoid organs, the correct positioning of cells, such as B and T cells, is dictated by the local production of chemokines by stromal cells, as well as the coordinated expression of chemokine receptors by migrating cells (5). The positioning of these cells and the migration during an immune response are critical for adequate interaction between them (38). Our results demonstrate that although B cell-expressed ADAM10 is dispensable for proper cellular positioning during LN and spleen development, ADAM10 is critical for the maintenance of the correct position of cells after immune stimulation. TNF-α is another ADAM10 substrate, and it has been implicated in the formation of primary B cell follicles and GCs. Unlike ADAM10β−/− mice, TNF-α-deficient mice have disrupted splenic architecture before immunization (39). The phenotype of ADAM10β−/− mice is also different from that seen with LT-deficient mice, because LT-deficient mice lack peripheral LNs and have severely disrupted splenic architecture. Moreover, draining and nondraining LNs from immunized mice had comparable levels of LT and TNF in WT and ADAM10β−/− mice (results not shown).

CCL21, CCL19, and CXCL13 have been shown to play a key role in inducing and maintaining normal cellular compartments (5). Draining LNs from ADAM10β−/− mice showed increased CCL21 expression. This increase in CCL21 was also confirmed at the protein level by immunohistochemistry. Interestingly, ADAM10β−/− mice had a larger area of CCL21 staining. The larger area of production is consistent with the aberrant T cell localization seen in draining LNs.

FDCs are important for GC development, Ig class switching, somatic hypermutation, and affinity maturation, as well as induction of recall responses (40–45). FDCs also secrete CXCL13 and are thus important for recruitment of CXCR5-expressing B cells and T cells into the follicle (46), and are important for the maintenance of B and T cell zones (38). The loss of B and T cell segregation and the lack of GCs in the draining secondary lymphoid tissues of ADAM10β−/− mice is thus consistent with fewer activated FDC networks. The changes in architecture correlate with the decrease in Tfh cells. Tfh cells provide B cell help via CD40L within GCs and are thus essential for PC differentiation, memory cell generation, and affinity maturation (6). Decreased Tfh cell number leads to decreased CD40L available to stimulate B cells, thus leading to diminished isotype switching. Decreased B cell activation is consistent with the phenotype we observed in the ADAM10β−/− mice and is the likely mechanism for the defect. These findings further support the idea that GC formation and Ab production are secondary to changes in architecture and decreased interaction between cognate B and T cells. Additional studies will be required to determine how B cell-expressed ADAM10 is functioning to cause this aberrant humoral response.

Secondary lymphoid organs are positioned such that they facilitate immune responses to foreign Ags. In states of chronic inflammation, lymphoid cell aggregates that have a similar organization to secondary lymphoid organs can develop; these structures are known as tertiary lymphoid structures. These structures have been described in patients with atherosclerosis and rheumatoid arthritis (38). Tertiary lymphoid organs have organized B cell compartments, which often include GCs, and T cell compartments, in which APCs can be detected. When tertiary organs develop at sites where autoantigen are continuously being presented, they may lead to unnecessary tissue destruction by facilitating the activation of autoreactive lymphocytes (38). Our results demonstrate that ADAM10 is critical for the structural maintenance of secondary lymphoid organs during active immune responses, and thus suggest a role for ADAM10 in the maintenance of tertiary lymphoid organs. Thus, targeting ADAM10 could be a potential therapy for diseases involving tertiary lymphoid tissues. Because complete loss of ADAM10 is lethal in utero (47), blockade of ADAM10 would need to be done locally. We have recently shown that intranasal administration of ADAM10 inhibitors reduced symptomology in the mouse asthma model without causing any obvious deleterious activities (48). Thus, it may be possible to use local ADAM10 inhibition in some autoimmune scenarios to decrease autoimmune symptoms.

In conclusion, examination of humoral responses in B cell-specific ADAM10-deficient mice has demonstrated that ADAM10 is critical for the maintenance of splenic and LN architecture during active immune responses. ADAM10β−/− mice have decreased numbers of Tfh cells, leading to diminished T cell help and impaired GC formation, affinity maturation, and class switch recombination. Therefore, B cell-expressed ADAM10 is required for proper humoral responses. Thus, B cell-expressed ADAM10 is essential for the maintenance of lymphoid structure after Ag challenge.

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References

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