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Notch Signaling Regulates Follicular Helper T Cell Differentiation

Floriane Auderset,∗ Steffen Schuster,∗† Nicolas Fasnacht, ‡ Manuel Coutaz,∗† Mélanie Charmoy,∗† Ute Koch, ‡ Stéphanie Favre,∗ Anne Wilson, § François Trottein, † James Alexander, † Sanjiv A. Luther,* H. Robson MacDonald,§ Freddy Radtke,‡ and Fabienne Tacchini-Cottier*‡

Follicular helper T (T FH) cells are specialized in providing help for B cell differentiation and Ab secretion. Several positive and negative regulators of T FH cell differentiation have been described but their control is not fully understood. In this study, we show that Notch signaling in T cells is a major player in the development and function of T FH cells. T cell–specific gene ablation of Notch1 and Notch2 impaired differentiation of T FH cells in draining lymph nodes of mice immunized with T-dependent Ags or infected with parasites. Impaired T FH cell differentiation correlated with deficient germinal center development and the absence of high-affinity Abs. The impact of loss of Notch on T FH cell differentiation was largely independent of its effect on IL-4. These results show a previously unknown role for Notch in the regulation of T FH cell differentiation and function with implications for the control of this T cell population. The Journal of Immunology, 2013, 191: 2344–2350.

F ollicular helper T (T FH) cells play a critical role in providing help for B cells to enhance germinal center (GC) formation, the generation of high-affinity Abs, and to mature into memory B cells and long-lived plasma cells (1, 2). The plasticity of different Th cell subsets has been recently shown (3), and it remains unclear whether T FH cells are a specific lineage. Following parasite infection, T FH cells produce most IL-4 or IFN-γ detected in draining lymph nodes (dLNs) (4–6). Although cytokines contribute to Th cell differentiation, they can be generated in the absence of cytokine signaling, demonstrating a role for other molecules in this process (reviewed in Ref. 7).

Notch proteins are a family of evolutionarily conserved transmembrane-bound receptors, which play crucial roles in binary cell fate decisions in many developmental systems. In mammals, four Notch receptors (Notch1–4) are activated by five transmembrane-bound ligands (Jagged 1 and 2 and Delta-like 1, 3, and 4). Interaction of Notch receptors with their ligands initiates the release of the active intracellular domain of Notch by proteolytic cleavages from the membrane, allowing its translocation into the nucleus. Once there, the intracellular domain of Notch forms a complex with recombination signal-binding protein-J, converting it from a repressor to an activator of transcription (8). There is increasing evidence revealing an important role for Notch signaling in the regulation of CD4+ Th cell differentiation or function in the periphery (9–11). However, the role of Notch in the differentiation of T FH cells has not previously been investigated.

In this study, we used mice carrying a T cell–specific deletion of Notch1 and Notch2 to show that Notch signaling in CD4+ T cells is an essential component for the differentiation of T FH cells. Impaired T FH cell development in the absence of Notch signaling correlated with strongly reduced numbers of GC B cells and decreased generation of high-affinity Abs during T-dependent immune responses. Collectively, our data reveal a Notch-specific contribution to T FH cell differentiation.

Materials and Methods

Mice

N1N2foxcre CD4-Cre mice (referred to hereafter as N1N2−/−) were previously described (12). N1N2foxcre littersmates were used as controls. All mice are on a C57BL/6 genetic background. Mice are from Charles River Laboratories (CD45.1), Harlan Olac (Bicester, U.K.; BALB/c), The Jackson Laboratory (Bar Harbor, ME; IL-4−/−), and Taconic (IL-4Rα−/−). CD4-Cre mice were also used as controls and gave similar results to N1N2foxcre mice (data not shown). All mice were bred and maintained under pathogen-free conditions in the Animal Facility at the Center of Immunity and Infection Lusanne (Epalinges, Switzerland). All experimental procedures with mice were approved by the Veterinary Office Regulations of the State of Vaud, Switzerland (authorization nos. 1266-3 and 1266-4).

Parasites and immunizations

Mice were infected s.c. with 3 × 106 Leishmania mexicana (MYNC/BZ/62/M379) amastigotes into the back rump or were immunized with 5000 Schistosoma mansoni eggs injected s.c. in the hind footpad. Mice were immunized s.c. with 25 μg per site 4-hydroxy-3-nitrophenyl acetyl (NP) conjugated to chicken γ-globulin (CGG) (NP67-CGG; Biosearch Technologies) in Montanide adjuvant ISA25 (25% in PBS; Seppic). dLN cells

The online version of this article contains supplemental material.

Abbreviations used in this article: BM, bone marrow; BTLA, B and T lymphocyte attenuator; dLN, draining lymph node; CGG, chicken γ-globulin; GC, germinal center; NP, 4-hydroxy-3-nitrophenyl acetyl; PD-1, programmed death-1; PP, Peyer’s patch; T FH cell, follicular helper T cell; WT, wild-type.

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from L. mexicana–infected mice were cultured with and without UV-irradiated parasites for 72 h. The cytokine contents of the cell supernatants were measured by ELISA. IL-4 and IL-5 cytokines were analyzed with OpiELIA kits (BD Biosciences), and IL-13 was analyzed with DuoSet kit (R&D Systems).

**Serum Ab quantification**

Ag-specific IgG1 and IgM Abs were quantified in sera by Ag-capture ELISAs and detected with biotinylated goat anti-mouse IgG1 and goat anti-mouse IgM (both from Caltag Laboratories). Total or high-affinity NP-specific IgG1 and IgM Ab levels were measured by ELISA using NP37-BSA or NP32-BSA (Biosearch Technologies).

**cDNA preparation and real-time PCR**

Total mRNA was extracted with the RNeasy mini kit (Qiagen), and cDNA was prepared as previously described (12). Quantitative real-time PCRs were done using SYBR Green and a LightCycler system (Roche). Primers used were previously described (4, 13, 14).

**Flow cytometry**

The following mAb conjugates were used to stain cells: CD4-PE-Cy5, -Alexa 700, -allophycocyanin-Alexa 750, -CD62L-PE, -CD44-allophycocyanin-Cy7, -CD25-Pacific Blue; TCRβ-PE-Cy5.5; CD8-FITC, -Pacific Blue, -allophycocyanin; B220-FTTC, -PE-Texas Red, -allophycocyanin, -PerCP-Cy5.5; programmed death-1 (PD-1)-PE; GL7-FITC; Fas-biotin; ICOS-PE-Cy5; and then stained with the following primary Abs: peanut agglutinin-biotin (Vector Laboratories), rat anti-CD4 (H129.19.6), rat anti-CD8 (SK1), and rat anti-syndecan biotin (BD Phar). Cells were isolated and frozen in optimum cutting temperature embedding compound (Saxon Rabbit Company). A total of 1 × 10⁷ donor cells were used, because these two receptors were shown to be selectively induced on activated peripheral CD4⁺ T cells (15). The frequency and number of CD4⁺ T cells (Fig. 1A) including naive CD4⁺ cells (CD62L⁺CD44low) and CD25⁺Foxp3⁺ regulatory T cells were normal in N1N2⁻/⁻ mice, comparable to those of control mice (Fig. 1B). The population of CD8⁺ T cells was normal as well (Fig. 1C). Of note, the lymph node architecture, with distinct T and B zones as well as the distribution of different hematopoietic and stromal cell populations in naive N1N2⁻/⁻ mice was comparable to that of control mice (Supplemental Fig. 1). Following infection with L. mexicana or injection of Schistosoma mansoni eggs, the total number of cells as well as the number of CD4⁺ T cells in the dLNs were similar in N1N2⁻/⁻ and control mice (Fig. 1D). Additionally, upon Ag stimulation, CD4⁺ T cell proliferation did not differ between both groups. No difference in T cell apoptosis was observed in N1N2⁻/⁻ and control Ag-stimulated dLN CD4⁺ T cells (Supplemental Fig. 2).

Having established that N1N2⁻/⁻ mice do not have defects in peripheral T cell populations, these and control mice were immunized with the well-defined T cell–dependent Ag NP conjugated to CGG in Montanide adjuvant. CD4⁺ TFH cells were identified in dLNs of immunized mice by FACS through their unique combined high expression of CXCR5, PD-1, BTLA, and the presence of the transcription factor Bcl6. Seven days after immunization, the percentage of T FH cells in dLNs of immunized N1N2⁻/⁻ mice was significantly lower than in control mice. N1N2⁻/⁻ T FH cells expressed reduced levels of the classical T FH cell markers CXCR5, PD-1, BTLA, and Bcl6, whereas they expressed normal levels of ICOS (Fig. 2A). To assess whether this decreased frequency of T FH cells was sustained following immunization, the kinetics of T FH cell development were measured in N1N2⁻/⁻ and control mice following NP-CGG immunization. At all time points, the frequency of T FH cells was markedly reduced in N1N2⁻/⁻ mice compared with control mice (Fig. 2B) whereas the number of T FH cells was significantly reduced from 7 d on (Fig. 2C).

To investigate whether Notch receptor signaling also contributes to T FH cell differentiation during physiological immune responses, N1N2⁻/⁻ and control mice were inoculated with L. mexicana amastigotes or with S. mansoni eggs, both parasites or parasite products inducing the differentiation of T FH cells. Indeed, infection with L. mexicana or inoculation of S. mansoni eggs led to the differentiation of T FH cells in control mice. In contrast, very few T FH cells were detectable in N1N2⁻/⁻ mice (Fig. 3A). Both the frequency and total number of T FH cells in dLNs of infected N1N2⁻/⁻ mice were strongly reduced (Fig. 3B). Of note, activation of N1N2⁻/⁻ non-T FH cells was not impaired, as shown by the presence of PD-1⁺CD4⁺ T cells. To visualize the presence of T FH cells in GCs, CD4⁺ T cell immunohistochemistry was performed in dLNs of L. mexicana–infected mice. Numerous CD4⁺ T cells were clearly visible in the GCs of N1N2lox/lox control mice, whereas only a few scattered CD4⁺ T cells were detectable over the primary B cell follicles of N1N2⁻/⁻ dLNs (Fig. 3C). Collectively, these data demonstrate that Notch receptors on T cells play a crucial role in the development of T FH cells during immune responses.

**Immunohistochemistry**

Poptailal or inguinal lymph nodes from naive mice, or from mice infected with L. mexicana, injected with S. mansoni eggs or with NP37-CGG were isolated and frozen in optimum cutting temperature embedding compound (Sakura Finetek). Sections of 7 μm were cut with a Leica cryostat and fixed in acetone. Sections were blocked with normal mouse and donkey serum and then stained with the following primary Abs: peanut agglutinin-biotin (Vector Laboratories), rat anti-B220 (RA3-6B2), rat anti-CD4 (M4412; BD Pharmingen), rat anti-CD8 (PK136). One day later, mice were lethally irradiated with a single dose of 900 rads. Donor bone marrow (BM) cells were isolated and incubated with anti-Thy1 culture supernatant (clone AT3b), DNase1, and rabbit complement (Saxon Rabbit Company). A total of 1 × 10⁷ donor cells were engraved i.v. into irradiated CD45.1 mice at a ratio of CD45.1/ control or N1N2⁻/⁻ of 1:3. Chimerism was analyzed after 8 wk of engrafment by FACS analysis.

**Statistical analysis**

Data were analyzed using the Student t test for unpaired data. When more than two variables were tested, the statistical analysis was performed using a one-way ANOVA followed by a Tukey multiple comparison test.
GC formation is impaired in immunized or infected Notch-deficient mice

Because T<sub>FH</sub> cells contribute to GC development and maintenance (1), we next compared GC B cells in NP-CGG–immunized N1N2<sup>−/−</sup> and control mice. Remarkably, relative to control mice, the number of GC B cells (Fas<sup>+</sup>GL-7<sup>+</sup>) was markedly decreased in N1N2<sup>−/−</sup> mice (Fig. 4A). Similarly, L. mexicana–infected or S. mansoni eggs–exposed N1N2<sup>−/−</sup> mice showed a strong reduction in the frequency of GC B cells (Fig. 4B). No detectable GCs were visible by immunohistology in dLNs of mice immunized with NP-CGG, with S. mansoni eggs or in L. mexicana–infected mice (Fig. 4C and data not shown).

TFH cells are essential to provide help to B cells for the production of high-affinity, class-switched Abs. The kinetics of NP-specific IgG1 secretion were quantified in the sera of NP-CGG–immunized mice, measuring high-affinity (NP<sup>4</sup>) and total (high and low) affinity (NP<sup>23</sup>) Abs. From 7 d on, with a maximum at 28 d after immunization, high-affinity serum IgG1 Abs were observed in control mice. In contrast, in line with the reduced number of GCs, hardly any high-affinity IgG1 Abs were detectable in sera from N1N2<sup>−/−</sup> mice (Fig. 4D). The absence of Notch receptor expression on T cells also affected the secretion of total NP-specific IgG1 Abs 28 d after immunization, consistent with the low number of GCs in these mice (Fig. 4D). NP-specific IgM Ab levels were not statistically different in immunized N1N2<sup>−/−</sup> and control mice. These data demonstrate a crucial role for Notch expression on T cells for GC-driven generation of high-affinity Abs following immunization with T-dependent Ags.

Notch affects T<sub>FH</sub> cell differentiation in a cell-autonomous manner independently of its effect on IL-4

N1N2<sup>−/−</sup> mice have a T cell–specific loss of function for Notch1 and Notch2, implying that impaired T<sub>FH</sub> cell differentiation in these mice is T cell autonomous. However, in contrast to the virtual absence of T<sub>FH</sub> cells detected in immunized or infected N1N2<sup>−/−</sup> mice, a small number of T<sub>FH</sub> cells were detectable in Peyer’s patches (PPs) of naive N1N2<sup>−/−</sup> mice (Fig. 5A). The high frequencies of T<sub>FH</sub> cells observed in PPs of naive wild-type (WT)
mice correlate with the very high activity of their GCs necessary to regulate the gut microbiota (16). The small proportion of T<sub>FH</sub> cells observed in N1N2<sup>−/−</sup> mice suggests that in the presence of sustained Ag stimulation and GCs, a small proportion of T<sub>FH</sub> cells may develop independently of Notch signaling. To verify this, we generated mixed BM chimeras. Lethally irradiated CD45.1 WT mice were reconstituted with either a 1:3 mix of CD45.1 WT/CD45.2 N1N2<sup>−/−</sup> BM or, as a control, of CD45.1 WT/CD45.2 N1N2<sup>lox/lox</sup> control BM. Eight weeks later, the chimeras were immunized with NP-CGG and the relative numbers of T<sub>FH</sub> and GC B cells derived from the CD45.2 (N1N2<sup>−/−</sup> or control) or CD45.1 (WT) donor marrow were determined. The CD45.2/CD45.1 T<sub>FH</sub> cell ratio was significantly decreased in the mixed WT/N1N2<sup>−/−</sup> BM chimeras compared with that of control WT/N1N2<sup>lox/lox</sup> chimeras. In contrast, the CD45.2/CD45.1 ratio of non-T<sub>FH</sub> cells was similar (Fig. 5B, 5C). The number of GC B cells derived from CD45.2 control or N1N2<sup>−/−</sup> BM was comparable in the two types of mixed BM chimeras (Fig. 5D), and similar levels of NP<sub>a</sub> high-affinity or NP<sub>p</sub> high- and low-affinity Abs were detected in sera of both types of mixed chimeras (data not shown). Collectively, these data demonstrate that the impaired T<sub>FH</sub> cell development in the absence of Notch is mainly T cell–intrinsic. Additionally, these data show that in the presence of WT T<sub>FH</sub> cells, GC B cells can develop normally in N1N2<sup>−/−</sup> mice.

Several studies showed that following infection with Th2-inducing parasites, most cells secreting IL-4 in dLNs were T<sub>FH</sub> cells (4–6). The direct effect of Notch on IL-4 transcription (17, 18) is distinct from that on the differentiation of T<sub>FH</sub> cells. Indeed, T<sub>FH</sub> cells can develop in the absence of IL-4 (4, 6) (Supplemental Fig. 3A, 3B), showing that the deficient development of T<sub>FH</sub> cells in N1N2<sup>−/−</sup> mice is not an indirect consequence of decreased IL-4 production.

Nonresponsiveness to IL-4 has been associated with the development of smaller GCs (6, 19). Accordingly, a decreased percentage of GC B cells was measured in the dLNs following NP-CGG injection in IL-4<sup>−/−</sup> and IL-4R<sup>α</sup>−/− mice. However, the frequency of GC B cells measured in immunized IL-4<sup>−/−</sup> and IL-4R<sup>α</sup>−/− mice remained significantly higher than that measured in dLNs of N1N2<sup>−/−</sup> mice, whereas no difference was observed between WT BALB/c and control C57BL/6 mice (Supplemental Fig. 3C–E).

Collectively, these data reveal that Notch signaling has a critical T cell–intrinsic effect on T<sub>FH</sub> cell development, which is distinct from its direct effect on IL-4.

**Notch affects the balance between Bcl6 and Blimp1 mRNA levels during the differentiation of Th subsets**

Thus far our data reveal a crucial role of Notch in T<sub>FH</sub> cell differentiation. The transcription factor Bcl6 is the master regulator of T<sub>FH</sub> cells, whereas Blimp1 is an antagonist of T<sub>FH</sub> cell differ-
entiation (20, 21). To further investigate the mechanism of Notch action in TFH cell differentiation, CD4+CXCR5 PD-1−/− (non-activated, non-TFH), CD4+CXCR5 PD-1int (pre-TFH), and CD4+CXCR5PD-1high PD-1int (TFH) cells from N1N2−/− and control mice were FACs sorted 7 d after immunization with NP-CGG (Fig. 6A). As expected, a marked increase in Bcl6 mRNA was measured in immunized control TFH cells and not in pre–TFH cells. In contrast, only the low Bcl6 mRNA level was induced in N1N2−/− TFH cells, in line with the very low number of these cells found in dLNs of N1N2−/− mice (Fig. 6B). All activated CD4+ T cells showed a significant increase in Blimp1 mRNA, with a markedly higher Blimp1 mRNA level in N1N2−/− activated pre–TFH cells (Fig. 6B).

The increased Blimp1 mRNA expression in this T cell population correlated with higher levels of IL-13 and IL-5 secretion by dLN T cells of immunized or infected N1N2−/− mice (data not shown and Supplemental Fig. 2C). The lower levels of secreted IL-21 and BTLA and that could be subdivided into GL7− non-GC TFH and GL7high GC TFH cells, only very few N1N2−/− PD-1high BTLAhighGL7high or GL7− TFH cells were detectable (Fig. 6D).

To further investigate the impact of Notch deficiency on the differentiation of TFH cells, non–TFH, pre–TFH, and TFH cells were similarly FACs sorted 7 d after the injection of S. mansoni eggs, which induces large number of TFH cells, and expression of several markers associated with TFH cells were analyzed by RT-PCR. A significant decrease in Bcl6 mRNA was observed in both N1N2−/− pre–TFH and TFH cells (Fig. 7A), in line with data obtained following NP-CGG immunization (Fig. 6B, 6C). High expression of IL-21 is another hallmark of TFH cells. Accordingly, TFH cells from control mice expressed high levels of IL-21 mRNA. In contrast, IL-21 mRNA expression was significantly impaired in N1N2−/− TFH cells (Fig. 7B). The levels of c-Maf mRNA and other transcription factors present in TFH cells were also reduced in the small population corresponding to N1N2−/− TFH cells, whereas the small decrease in BATF mRNA observed in N1N2−/− TFH cells was not statistically significant from that of control TFH cells (Fig. 7C). Thus, the few TFH cells present in dLNs of N1N2−/− mice do not express the characteristic array of markers defining differentiated TFH cells.

Additionally, the phenotype of pre-TFH cells was altered in N1N2−/− dLNs, with a markedly increased level of Blimp1 mRNA (Fig. 7D), as observed in NP-CGG–immunized mice (Fig. 6B). The frequency and number of CD4+PD-1int pre–TFH cells were similar between N1N2−/− and control mice, but the expression of CXCR5 was already decreased in that population for both NP-CGG– and S. mansoni eggs–immunized mice (Fig. 7E, 7F).

These results show that under physiological conditions, very few TFH cells develop in N1N2−/− dLNs and that these TFH cells lack most of the classical TFH cell markers.

Discussion

In this study we have demonstrated that following immunization with T-dependent Ags or after exposure to Th2-inducing parasites, expression of Notch1 and Notch2 on T cells is essential for the differentiation of TFH cells. Consequently, markedly reduced GC development as well as impaired maturation of high-affinity Ab-producing B cells was observed. This defect was shown to be T cell–intrinsic using mice with selective inactivation of Notch1 and Notch2 in their T cells, as well as mixed BM chimeras.

Increasing evidence implicates Notch signaling as a player in the differentiation of functional CD4+ Th subsets. The impact of Notch signaling may affect Th cell differentiation and/or function (9–11). Using mice with a T cell–specific deletion of recombination signal-binding protein-J, Notch1, and Notch2, or with conditional expression of dominant-negative MAML1, an essential role for Notch in the development of a Th2 response was reported (22, 24, 25). Of note, in these studies, only IL-4 and its related isotype switching were measured as a readout for Th2 cell differentiation. Activation of IL-4 in TFH cells was recently shown to be mainly dependent on a conserved IL-4 enhancer (CNS2) (26, 27), and Notch intracellular domain was previously shown to bind selectively to CNS2 in a reporter assay (17, 18, 28). Only very few TFH cells developed in N1N2−/− mice following Th2-inducing stimuli, and we could not detect significant IL-4 levels in dLN T cells, suggesting that the absence of Notch signaling on T cells...
Similarly, a small percentage of TFH cells could also develop in the absence of strong TCR signaling and high cytokine levels. Thus, a small proportion of TFH cells may develop independently of IL-4 secretion by CD4+ T cells suggests that the early IL-4 needed for Th2 differentiation originates from other cell types, as previously reported for Th2 cells. In the presence of a functional GC, in mixed BM chimeras, a small number of Notch-deficient T cells developed into TFH cells. Similarly, a small percentage of Th2 cells could also develop in the PPs of naïve N1N2−/− mice that have a high number of GCs resulting from T-independent and T-dependent responses to gut microflora. During in vitro differentiation, the need for Notch signaling in Th cell differentiation can be bypassed in the presence of strong TCR signaling and high cytokine levels. Thus, a small proportion of TFH cells may develop independently of the need of Notch when sustained strong Ag signaling and/or chronic GCs are present.

We show that Notch signaling regulates the balance between Bcl6 and Blimp1 mRNA expression. Bcl6 is the master transcription factor of TFH cells, and its expression is repressed by Blimp1, which is a transcription factor expressed by other Th effector cells. These two transcription factors regulate each other by Blimp1, which is a transcription factor expressed by other Th effector cells. These two transcription factors regulate each other and determine commitment to either TFH or other Th effector cells. These two transcription factors regulate each other and determine commitment to either TFH or other Th effector cells. These two transcription factors regulate each other and determine commitment to either TFH or other Th effector cells.

**FIGURE 6.** Notch expression on T cells affects the Bcl6/Blimp1 ratio. (A) PD-1highCXCR5+CD4+ TFH cells, PD-1intCXCR5+CD4+ non-TFH cells, and PD-1–CXCR5–CD4+ nonactivated cells were FACS sorted 7 d after NP-CGG immunization according to the gates. (B) Bcl6 and Blimp1 mRNA levels were analyzed in the three sorted populations by RT-PCR and normalized to HPRT mRNA expression. Results are presented as arbitrary units ± SEM for n ≥ 3 mice/group. (C) Bcl6 expression was analyzed by intracellular staining in PPs and dLN of naïve and NP-CGG–immunized N1N2−/− and control mice, respectively. The mean fluorescence intensity (MFI) of Bcl6 expression is represented in histograms ± SEM for n = 3 mice/group. (D) GL7 expression was analyzed by flow cytometry in PD-1highCXCR5+CD4+ TFH cells (green), PD-1intCXCR5+CD4+ non–TFH cells (blue), and PD-1–CXCR5–CD4+ nonactivated cells (red) of S. mansoni eggs–immunized N1N2−/− and control mice. Representative FACS plots of n = 5 mice are given. All data are representative of at least two independent experiments. *p < 0.05.
both c-Maf and Bcl6 mRNA levels; however, only a slight reduction of BATF mRNA levels was observed, suggesting that the control of Notch signaling on $T_{FH}$ cell differentiation does not act directly via the regulation of BATF expression. The exact Notch targets involved remain to be determined.

We further show that the few $T_{FH}$ cells detectable in N1N2−/− mice lack the main classical markers of $T_{FH}$ cells such as surface expression of CXCR5, high levels of PD-1, BTLA, and GL7 surface molecules, high levels of IL-21 mRNA and expression of Bcl6 and c-Maf transcription factors. Deficiency in IL-21 has been reported to have mild or no effect on $T_{FH}$ cell differentiation, but IL-21 contributes to affinity maturation and GC formation (34–37). Absence of IL-21 results in a 50% reduction of GC B cell number whereas absence of $T_{FH}$ cells results in total loss of GC (20, 21, 23). Notch 1 and notch 2 surface expression during T cell development and activation revealed a novel monoclonal antibodies. J. Immunol. 183: 7212–7222.


