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Notch Ligands Expressed by Follicular Dendritic Cells Protect Germinal Center B Cells from Apoptosis

Sun-Ok Yoon,* Xin Zhang,* Paul Berner,* Bianca Blom, † and Yong Sung Choi1*

The Notch signaling pathway is one of the most conserved mechanisms to regulate cell fate in many tissues during development and postnatal life. In the immune system, Notch signaling regulates T and B cell development and modulates the differentiation of T and B cells. In this study, we investigated the functional roles of Notch signaling in human B cell differentiation within the germinal center (GC). Notch ligands, Delta-like 1 (Dll1) and Jagged 1 (Jg1), are expressed by follicular dendritic cells (FDC) but not by B cells in the GC, while GC-B cells express the Notch receptors, Notch1 and Notch2. The blockade of Notch signaling pathways using a γ-secretase inhibitor, DAPT (N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester), reduces the survival of GC-B cells in the presence of FDC/HK cells. Jg1 has a dominant effect on GC-B cell survival mediated by Notch signaling. Furthermore, Notch cooperates with another anti-apoptotic factor, BAFF/Blys produced by FDC to support GC-B cell growth. Taken together, our data shows the important role of Notch signaling provided by FDC in the survival of GC-B cells in vitro. The Journal of Immunology, 2009, 183: 352–358.

Notch signaling regulates cell fate during development and differentiation in many cell lineages including lymphocytes. Activation of Notch receptors on the cell surface requires direct contact with adjacent cells expressing Notch ligands (1, 2). Notch receptors activated by interaction with ligands induce the proteolytic cleavage of Notch, allowing the intracellular portion of Notch (ICN)2 to translocate to the nucleus and interact with the DNA-binding transcription factor CBFI that modulates the expression of downstream target genes.

It has recently been reported that Notch signaling plays critical roles in B cell development (3). Loss-of-function studies with Notch signaling components [Notch2, CSL, Delta-like 1 (Dll1), or Mastermind-like 1 (MAML1)] disrupt peripheral lymphoid tissue, resulting in the selective failure to generate marginal zone B cells (4–7). In addition, Notch signaling has recently been shown to affect activation and differentiation of mature B cells to Ab-secreting cells in murine experimental models (8, 9). These previous in vitro experiments, using mature murine B cells and exogenous Notch ligand expressing cell lines, reveal the important contribution of Notch signaling to the generation of plasma cells (PC). However, functional roles of Notch signaling in human B cell differentiation in the germinal center (GC) are not clear.

The GC is a specialized microenvironment where Ag-activated B cells undergo clonal expansion, somatic hypermutation, Ig class switch recombination, and differentiate into either memory B cells or PC secreting high-affinity Abs (10–12). The cellular and genetic events during the GC reaction occur through both contact-dependent and independent interactions of Ag-activated B cells with follicular dendritic cells (FDC) and activated T cells. Although the cellular and molecular interactions between T and B cells have been extensively investigated for three decades, the functional roles of FDC have only recently been revealed (13, 14).

FDC and T cells have essential but distinct functional roles in GC-B cell differentiation (15, 16). While T cells induce GC-B cell differentiation by providing CD40L and cytokines, FDC protect GC-B cells from apoptosis and support proliferation by providing FDC-signaling molecules (FDC-SMs) (14, 16). In the very early stages of the GC reaction, both FDC and CD40L from T cells are essential for GC-B cell survival (15). More than 90% of GC-B cells die within 24 h in culture in the absence of CD40L and FDC. Whereas apoptosis of GC-B cells is transiently prevented by anti-CD40 or CD40L (17, 18), FDC sustain the survival of GC-B cells for longer than 24 h (17, 19–21). However, FDC-SMs required for GC-B cell survival have not been thoroughly characterized. To identify FDC-SMs, we have established an FDC line, HK, from human tonsils that has FDC functions (20, 22). HK cell line provides a unique in vitro experimental model that mimics the GC reaction in vivo (15, 23). GC-B cells can be cultured on HK cells with CD40L and T cell cytokines for more than 14 days (23). Using this in vitro experimental model where we were able to study the functional roles of FDC in molecular terms (14), we investigated the functions of Notch signaling in the GC.

Here, we show that Notch ligands, Dll1 and Jg1 are expressed by human FDC whereas GC-B cells express Notch receptors, Notch1 and Notch2. The Notch signaling in the interaction between FDC and GC-B cells protects GC-B cells from apoptosis in the very early stages of GC-B cell differentiation. Of the two Notch ligands expressed by FDC, Jg1 appears to have a dominant role in GC-B cell survival.

Materials and Methods

Antibodies and reagents

Mouse anti-human Dll1 and goat anti-human Jg1 were purchased from R&D Systems. Goat anti-human Jagged 2 (Jg2) was obtained from Santa

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1 Abbreviations used in this paper: ICN, intracellular portion of Notch; GC, germinal center; FDC, follicular dendritic cell; PC, plasma cell; FDC-SM, FDC signaling molecule; Dll1, Delta-like 1; Jg1, Jagged 1; Jg2, Jagged 2; GSI, γ-secretase inhibitor; DAPT, N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester; PI, propidium iodide.

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Cruz Biotechnology. Other Abs used in this work were PE-conjugated anti-8D6 (16); FITC-conjugated anti-CD20, PerCP-conjugated anti-CD20, antilymphocytic conjugated anti-CD38, and FITC- or PE-conjugated goat anti-mouse Ig (BD Biosciences); FITC- or PE-conjugated donkey anti-goat IgG (Jackson Immunoresearch Laboratories).

Cytokines used were IL-2 (Hoffman-La Roche), IL-4 (a gift from Schering-Plough, Union, NJ), and IL-21 (Biovision). Soluble human CD40L was provided by Dr. R. Armitage (Angen Corporation, Seattle, WA). CSF and DMSO were obtained from Sigma-Aldrich. DAPT (N-[N-(3,5-difluorophenacetyl]-l-alanyl]-S-phenylglycine t-butyl ester) was purchased from Calbiochem.

Preparation and culture of B cell subsets

GC-B cells and naive B cells were isolated from tonsillar B cells as described previously (15, 20). GC-B cells (2 × 10⁶ cells/well) were cultured in 96-well plates containing irradiated HK cells. DII1-OP9, Jg1-OP9, or OP9 control (2 × 10⁶ cells/well; 5000 rad), CD40L (100 ng/ml), IL-2 (30 U/ml), and IL-4 (50 U/ml) or IL-21 (10 ng/ml) in the absence or presence of DAPT. The culture medium was IMDM (Irving Scientific) supplemented with 10% FCS (Invitrogen), 2 mM glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin (Irving Scientific). Naive B cells were cultured using the same condition as GC-B cells but without HK cells.

For apoptosis assays, GC-B cells were cultured with CD40L and HK, DII1-OP9, Jg1-OP9, or OP9 control cells in the absence or presence of DMSO (25 μM), DAPT (25 μM), or anti-IL-6 (10 μg/ml) for 24 h. The cells were collected and stained with Annexin V-FITC and propidium iodide (PI; BD Biosciences) according to the protocol provided by the manufacturer.

For cell division experiments, GC-B cells were labeled with CFSE (5 μM in PBS) at 37°C for 10 min. FCS was added to stop staining, and then labeled cells were washed with culture medium. After culture with 25 μM DAPT or DMSO, the CFSE intensity was measured by FACSCalibur.

Flow cytometry and ELISA

Cells were stained with PerCP-conjugated anti-CD20 and antilymphocytic conjugated anti-CD38 as previously described (15). In brief, cells were incubated with the appropriate concentration of mAbs for 15 min at 4°C. After washing with PBS containing 0.2% BSA and 0.1% sodium azide, the cells were fixed with 1% parformaldehyde and analyzed by flow cytometry. The amount of IgG, IgM, and IgA in the culture supernatants was determined on 1.5% agarose gels and visualized by ethidium bromide staining. The specificity of amplification was validated by running a melting curve using slow denaturation of the PCR products. The primer sequences used are as follows; Deltex1 (forward: 5'-CCC TCC AAA ATC AAG TGG GG-3') (27), Hes1 (forward: 5'-CCC TCT CCC CAC CTC TAA GTT G-3') (29), Notch3 (forward: 5'-GCC AGA CCA TCA GCG AGT CAG C-3') (27), Notch2 (forward: 5'-CCA CCC GCG ACT CGC GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3'), reverse: 5'-GGG GTA AUA AGG AGG CTG GGC CTA-3').

Quantitative real-time PCR reactions were performed in a 10-μl volume containing 25 ng of cDNA, QuantiTect SYBR Green PCR Master Mix (Qiagen), and 2.5 μM of each gene-specific primer. The DNA Engine Opticon system with PTC-200 DNA Engine cycler and CFD-3200 Opticon Detector (MJ Research) were used with the following program: 95°C for 15 min, followed by 40 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 1 min. After cycling, the specificity of amplification was validated by generating a melting curve using slow denaturation of the PCR products. The primer sequences used are as follows; Deltex1 (forward: 5'-TGG GCC GCA AGA CCC AGA A-3'), reverse: 5'-TCC TCT CCA TCT CTT TGG GTG AAG G-3'), reverse: 5'-TAA GCC CCT TCT CCA AGG AGG TGA AAG G-3'), reverse: 5'-TAA GCC CCT TCT CCA AGG AGG TGA AAG G-3'), reverse: 5'-TAA GCC CCT TCT CCA AGG AGG TGA AAG G-3'), reverse: 5'-TAA GCC CCT TCT CCA AGG AGG TGA AAG G-3'). Ct values were analyzed with the ΔΔCt method using β2-microglobulin expression for normalization.

Statistical analysis

Statistical analysis and graphic presentation were conducted with GraphPad Prism 4.0. Results are presented as means of triplicate assays plus SEM. The statistical significance of differences was determined by Student’s t test; p < 0.05 was considered significant.

Results

FDC express Notch ligands and GC-B cells express Notch receptors

Since it was not known whether Notch signaling plays a functional role in human B cell differentiation in the GC, we first determined whether Notch ligands are expressed in tonsillar tissue. As shown in Fig. 1, DII1 and Jg1 are expressed in the GC whereas Jg2 is expressed only outside the GC. To identify the cellular source of DII1 and Jg1 in the GC, FDC-specific 8D6 mAb (16, 30) and B cell-specific anti-CD20 mAb were used to co-stain with anti-DII1 mAb or anti-Jg1 mAb, respectively. Both anti-DII1 mAb and anti-Jg1 mAb (green) co-stained with anti-8D6 mAb (red; co-staining, yellow; Fig. 1, A and B), but not with anti-CD20 mAb (supplementary Fig. S1A, A and B),3 suggesting that 8D6-positive FDC, but not B cells, produce Notch ligands, DII1 and Jg1. The stainings were specific because anti-DII1 and anti-Jg1 Abs stained DII1 and Jg1 expressing OP9 cells, respectively (supplementary Fig. S1C and S1D) and any specific signals were not detected on tonsillar tissue sections when stained with corresponding control Abs (Fig. 1). At the same time, a FDC line, HK cells, expressed DII1 and Jg1 in a single cell line derived from FDC express the identical type of Notch ligands.

The expression of Notch ligands by FDC suggests that Notch signaling may have a biological function in the GC-B cells because GC-B cells require FDC/HK for survival, proliferation, and differentiation. Thus, the expression of Notch receptors was examined in GC-B cells. mRNAs specific for Notch1 and Notch2 were expressed in ex vivo GC-B cells but not Notch4 (Fig. 1E). Notch3 expression was detectable but negligible. The same Notch receptors were expressed in tonsillar naive B cells and human peripheral blood B cells as reported previously (31).

3 The online version of this article contains supplemental material.
Blockade of Notch signaling reduces GC-B cell growth

Since Notch is activated through binding to its ligand on adjacent cells (1), we determined whether Notch ligands on FDC/HK cells have an influence on GC-B cell growth and differentiation. GC-B cells were cultured with CD40L, IL-2, and IL-4 in the presence of HK cells to induce cellular growth and expansion, as described in Materials and Methods (32). The effects of the Notch signaling blockade were investigated using a well-accepted γ-secretase inhibitor (GSI), DAPT that inhibits activation of the Notch signaling pathway by blocking ligand-induced cleavage of Notch (33).
When various amounts of DAPT were added in culture, GC-B cell growth was inhibited in a dose-dependent manner (Fig. 2A). A similar inhibition was observed when GC-B cells were cultured with IL-21, which induces differentiation of GC-B cells to Ab-secreting cells (Fig. 2B) (34). The addition of DAPT to the culture reduced Ab production of all isotypes (IgA, IgM, and IgG). The extent of the reduction was proportional to the amount of DAPT added (Fig. 2C). The reduction in Ab production by DAPT appeared to be the consequence of reduced GC-B cell growth because the percentage of CD20lowCD38high plasmablasts generated remained the same regardless of DAPT dose (Fig. 2D). This data suggests that Notch signaling is required for GC-B cell proliferation that precedes differentiation.

To confirm that DAPT specifically blocked Notch signaling activated by Notch ligands from FDC/HK cells, the inhibitory effect of DAPT was examined on naive B cells that do not require FDC/HK cells for survival and growth (35). DAPT had no inhibitory effect on the survival or proliferation of naive B cells whether cytokines were added or not (Fig. 3A). In addition, DAPT did not exhibit any cytotoxic effects on HK cells in culture (Fig. 3B), ruling out the possibility that inhibition of GC-B cell growth resulted indirectly from the cytotoxic effect of DAPT on HK cells. Since GSI is known to inhibit a diverse array of signaling events besides Notch signaling (36), we further determined whether DAPT affected specific transcriptional activation by Notch receptors. Quantitative real-time PCR measuring transcription of downstream targets of Notch signaling, Hes1 and Deltex1 (2) revealed that DAPT reduced both Notch-activated transcripts by more than 70% in GC-B cells cultured for 24 h (Fig. 3C).

**Notch signaling blockade affects GC-B cell survival**

Ag-activated B cells entering the GC appear to undergo a sequential process of survival, proliferation, and differentiation (12). Since it had not been determined which of these cellular processes were mediated by Notch signaling, we cultured GC-B cells with CD40L and HK cells for 24 h to examine the effect of Notch signaling on cell survival. Although less than 10% of seeded GC-B cells survived after 24 h in the absence of CD40L and HK cells, more than 70% of GC-B cells survived in the presence of CD40L and HK cells, which is greater than CD40L or HK cells alone (Fig. 4). The blockade of Notch signaling by DAPT decreased Annexin V− GC-B cells by 25% compared with that of control (i.e., GC-B cells cultured with DMSO). Inhibition by DAPT is specific because the addition of a neutralizing Ab against IL-6, a PC differentiation factor produced by HK cells (22), did not affect GC-B cell survival. The result is reproducible in similar experiments using three different donors as summarized in Table I, suggesting that Notch signaling from HK cells protects GC-B cells from apoptosis.

Since GC-B cells surviving apoptosis undergo proliferation and differentiation in order, we determined whether Notch signaling also has a direct effect on the cellular proliferation of GC-B cells. GC-B cells were labeled with CFSE and cultured with DMSO or DAPT in the presence of HK cells, CD40L, IL-2, and IL-4 to analyze the effect of Notch signaling on cell cycle progression. There was no difference in cell division profiles between the culture with DAPT and DMSO (supplementary Fig. S2). A similar

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**FIGURE 3.** DAPT effect on GC-B cells is specific to Notch signaling. DAPT does not affect the viability of either naive B cells (A) or HK cells (B). A. Tonsillar naive B cells (2 × 10^5 cells/24-well) were cultured with 25 μM DAPT or DMSO for 3 days in the presence of medium, IL-2 plus IL-4 plus CD40L, or IL-21 plus CD40L. Viable cells were counted by trypan blue exclusion assay. B. HK cells (0.5 or 1 × 10^5 cells/6-well, as indicated) were cultured with 25 μM DAPT or DMSO for 3 days. Cells were harvested at the end of the culture and recovered live cells are presented as percentages, compared with the control culture containing DMSO (100%). C. Quantitative real-time PCR analysis on the expression of Hes1 and Deltex1, downstream targets of Notch in GC-B cells after culturing with 25 μM DAPT or DMSO for 24 h. The relative fold changes of each transcript are shown in comparison to the level of the transcript in DMSO-treated GC-B cells, which is assigned the value 1.

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**FIGURE 4.** DAPT causes apoptosis of GC-B cells. Annexin V-FITC and PI binding were measured by flow cytometry after culturing GC-B cells for 24 h as indicated. Typical dot plots are shown.
result was observed when GC-B cells were cultured with IL-21, which induces more vigorous GC-B cell proliferation than IL-2 and IL-4. This data suggests that Notch signaling is required for the survival of GC-B cells but has no direct effect on proliferation and differentiation of the surviving cells.

**Jg1 has a dominant effect on GC-B cell survival**

To assess the effect of each Notch ligand on GC-B cells, we cultured GC-B cells in the presence of OP9 stromal cells transduced with control OP9 cell line, which is assigned the value 1. Significant differences are indicated (\(p < 0.01\), \(p < 0.05\); NS, not significant).

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Day</th>
<th>Live Cell (%) (Annexin-V - PI)</th>
<th>Apoptotic Cell (%) (Annexin-V - PI)</th>
<th>Dead Cell (%) (Annexin-V - PI)</th>
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<td>88.2</td>
<td>9.2</td>
<td>2.1</td>
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<tr>
<td></td>
<td>Day 1, medium</td>
<td>11.7</td>
<td>61.6</td>
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<td></td>
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<td>18.8</td>
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</table>

**FIGURE 5.** Jg1 has a dominant effect on GC-B cell survival. GC-B cells (2 \(\times 10^4\) cells/well) were cultured in 24-well plates with irradiated HK, Dll1-OP9, Jg1-OP9, or OP9 control cell lines (2 \(\times 10^4\) cells/well, 5000 rad) in the presence of CD40L, IL-2, and IL-4 (A) with 25 \(\mu\)M DAPT or DMSO (B). After 4 days of culture, viable cells were counted, and the increased cell recovery was presented as percentages compared with the control OP9 culture (100%). C, Annexin V-FITC and PI binding were measured by flow cytometry after culturing GC-B cells in the presence of HK, Dll1-OP9, Jg1-OP9, or OP9 control cell lines and CD40L with 25 \(\mu\)M DAPT or DMSO for 24 h. PI- dead cells were excluded from analysis and apoptotic Annexin V+ cells shown as percentage. D, Real-time PCR analysis of Hes1 and Deltex1 was performed on GC-B cells after 24 h of coculture with Dll1-OP9, Jg1-OP9, or control OP9 cell lines in the presence of CD40L, IL-2, and IL-4. The relative fold changes of each transcript are shown in comparison to the level of the transcript in GC-B cells cultured with control OP9 cell line, which is assigned the value 1. Significant differences are indicated (\(**, p < 0.01\); *, \(p < 0.05\); NS, not significant).

**FIGURE 6.** Notch signal cooperates with another anti-apoptotic factor, BAFF/Blys for GC-B cell growth. GC-B cells (2 \(\times 10^4\) cells/well) were cultured in 24-well plates in the presence of irradiated HK cells (2 \(\times 10^4\) cells/well, 5000 rad) and CD40L, IL-2 and IL-4 (A) or IL-21 (B) with anti-BAFFR (10 \(\mu\)g/ml), DAPT (25 \(\mu\)M), or both for the indicated time periods. Cells were harvested and counted by trypan blue exclusion assay. Significant differences are indicated (\(**, p < 0.01\); *, \(p < 0.05\); NS, not significant).
Anti-BAFFR further decreases GC-B cell growth in combination with DAPT

BAFF is an anti-apoptotic factor for B cells (37–39). Since FDC/HK cells produce BAFF that protects GC-B cells from apoptosis (40), we examined whether Notch could cooperate with BAFF to support GC-B cell growth. Whereas blocking BAFF signaling with anti-BAFFR decreased the recovery of GC-B cells by 10%, the combination of DAPT and anti-BAFFR decreased GC-B cell recovery by 55–65%, which was greater than DAPT alone (35–50%) (Fig. 6). This additive effect of DAPT and anti-BAFFR was not affected by T cell cytokines added to the culture (i.e., IL-2 plus IL-4 or IL-21), which induced the differentiation of GC-B cells, suggesting that both Notch and BAFF signaling are required for survival and growth before differentiation.

Discussion

We report that Notch signaling pathways play important functional roles in the proliferation and differentiation of Ag-activated B cells in the GC. We first identified the cellular source of Notch ligands and receptors in tonsillar tissue. Notch ligands, Dll1 and Jag1, are expressed by the FDC network in the GC of tonsillar tissue while GC-B cells do not express Notch ligands but receptors, Notch1 and Notch2. Since an FDC line, HK, express the same ligands as primary FDC, we are able to investigate the functional roles of Notch signaling in our in vitro experimental model. GC-B cells can be cultured on HK cells with CD40L and T cell cytokines for longer than 14 days in our culture system (23) whereas more than 90% of GC-B cells undergo spontaneous apoptosis within 24 h without FDC and T cell help (17, 19–21). Thus, our experimental model allows us to investigate the effect of Notch signaling on GC-B cell differentiation.

Ag-activated B cells entering the GC appear to undergo a sequential process of survival, proliferation, and differentiation to generate memory B cells and plasma cells (12). Our experimental results show that the initial survival of GC-B cells is a critical checkpoint that precedes proliferation and differentiation. The blockade of Notch signaling by specific inhibitor DAPT decreases the survival of GC-B cells but does not directly decrease proliferation and differentiation of surviving GC-B cells. Furthermore, we find that Jag1 but not Dll1 is the functional ligand for GC-B cell survival using Jag1- or Dll1-expressing OP9 stromal cells. Since GC-B cells express both Notch1 and Notch2, it is not conclusive which Notch receptor Jag1 interacts with to deliver survival signals to GC-B cells. Experiments using Notch ligand expressing cell lines also confirm that FDC/HK cells produce other factors required for GC-B cell survival and growth besides Notch ligands because the recovery of GC-B cells with HK cells is consistently higher than that with Jag1-OP9. Notch ligands expressed by FDC/HK may cooperate with other FDC factors to support GC-B cell growth. Indeed, GC-B cell recovery is decreased further when Notch signaling is blocked together with known anti-apoptotic factor BAFF produced by FDC/HK cells (40). Therefore, it is of interest to investigate how FDC signaling molecules are integrated to regulate the survival and growth of Ag-activated B cells in the GC.

The functional role of Notch signaling in murine B cell proliferation and differentiation has been reported recently (8, 9). However, the observations made in mouse B cells differ from our data with human GC-B cells in several aspects. First, Notch signaling enhanced the proliferation of the cells upon anti-CD40 stimulation through cell division in mouse B cells instead of cell survival (8, 9). This discrepancy may be explained by the difference in B cell subsets, purified human GC-B cells vs murine spleen B cells. The former cells die in culture without FDC whereas the latter survive without FDC. Second, only Jag1 has a positive effect in enhancing GC-B cell survival although human FDC in the GC expressed both Dll1 and Jag1. In contrast, Dll1 is the only Notch ligand detected in the FDC area of follicles in mouse spleen to enhance B cell proliferation (8, 9). Last, Notch signaling plays important roles in generation of plasma cells in the murine system (8, 9). Two Notch ligands, Jag1 and Dll1, have opposite effects on plasma cell generation through the same receptor Notch1, which was inducible upon activation with LPS (9). However, Notch signaling does not directly affect PC generation in our GC-B cell culture (i.e., 3–4 day short-term culture), as evidence by the following observations: 1) there is no decrease in percentage of plasmablasts generated from GC-B cells when Notch signaling is blocked by DAPT under culture conditions that induce PC differentiation (i.e., CD40L and IL-21), as assessed by CD20 and CD38 expression; and 2) there is no difference in plasmablast generation when GC-B cells are cultured with Jag1 or Dll1 expressing cell lines or control OP9 cell line (data not shown).

Notch signaling occurs when Notch-expressing cells interact with adjoining cells expressing Notch ligands (1, 2). Although different types of cells in the GC may express Notch and Notch ligands, our results show that presentation of Jag1 by FDC to GC-B cells rescue GC-B cells from spontaneous apoptosis using in vitro culture system. This interaction is likely to occur in vivo because FDC are found in close contact with B cells within the GC (10).

Our observations may have significant implication in treating B cell lymphomas. The majority of B cell lymphomas originates from the GC (41) and remains dependent on the GC microenvironment-like GC-B cells (42). Since it has recently been speculated that stromal microenvironments may contribute to the development of drug resistance in B cell lymphomas during treatment with rituximab (43), targeting the Notch signaling pathway deserves consideration as a candidate for treatment of B cell lymphomas in combination with the current therapy.

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Disclosures

The authors have no financial conflict of interest.

References

Supplemental Legends

Figure S1-A and B. B cells in the GC do not express either Dll1 or Jg1. Cryosections of tonsils were double-stained with anti-Dll1 (red fluorescence, A) or anti-Jg1 (red fluorescence, B) and anti-CD20 (green fluorescence, A and B). Figure S1-C and D. Anti-Jg1 and anti-Dll1 antibodies are specific. Jg1 expressing OP9 cells (C) and Dll1 expressing OP9 cells (D) were stained with anti-Jg1 Ab and anti-Dll1 Ab, respectively. At the same time, isotype control Abs were used in the place of anti-Jg1 Ab and anti-Dll1 Ab for negative controls.

Figure S2. DAPT does not affect GC-B cell proliferation. GC-B cells were labeled with CFSE (5 µM) and then cultured for 4 days with 25 µM of DMSO or DAPT in the presence of HK cells, CD40L, IL-2 and IL-4 (A) or IL-21 (B). CFSE profiles of harvested cells at day 1 and day 4 were compared by FACS analysis.
Supplementary Figure 2