Toll-like receptor 9 activation induces expression of membrane-bound B-cell activating factor (BAFF) on human B cells and leads to increased proliferation in response to both soluble and membrane-bound BAFF

Eman Y. Abu-Rish¹, Yassine Amrani¹,² and Michael J. Browning¹,³

Abstract

Objectives. Activation of TLR7 and TLR9 and high serum levels of BAFF have been implicated in the pathogenesis of SLE. However, little is known about the effects of TLR9 activation on BAFF expression by human B cells. We investigated the effect of the TLR9 agonist, CpG-ODN 2006, on the expression of BAFF and its receptors BAFF-R, TACI and BCMA, in isolated B cells from healthy donors.

Methods. We used RT-PCR, flow cytometry and ELISA to investigate the expression of BAFF, and flow cytometry for BAFF-R, TACI and BCMA. Functional assays assessed the responses of resting and CpG-ODN-activated B cells to exogenous soluble and membrane-bound BAFF.

Results. CpG-ODN did not induce BAFF secretion, but increased expression of membrane-bound BAFF on B cells. CpG-ODN also induced the expression of TACI and BCMA, but did not up-regulate BAFF-R expression. In functional studies, CpG-ODN sensitized human B cells to proliferate in response to exogenous BAFF. This effect was inhibited by a blocking antibody against BAFF-R, but was not inhibited by anti-TACI or anti-BCMA antibodies. Membrane-bound BAFF, induced by CpG-ODN, co-stimulated the proliferation of B cells stimulated with anti-IgM in a manner that was dependent on the expression of surface BAFF on the CpG-ODN-treated B cells.

Conclusion. TLR9 activation induces expression of membrane-bound BAFF on human B cells and leads to increased proliferation in response to both soluble and membrane-bound BAFF. These data extend our understanding of the role of TLR9 activation on human B cells and provide insights into the mechanisms by which TLR9 may participate in the pathogenesis of SLE.

Key words: TLR9, BAFF, BAFF-R, TACI, BCMA, CpG-ODN, B cell.

Introduction

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that recognize conserved molecular motifs on pathogens and direct the immune system to respond to microbial infections. TLR9 recognizes unmethylated DNA sequences, commonly found in bacterial and viral DNA and in synthetic unmethylated CpG oligodeoxynucleotides (CpG-ODN) [1–3]. In humans, TLR9 is expressed predominantly on B cells and plasmacytoid dendritic cells (pDCs) [4–7]. In B cells, the effects of CpG-ODN-mediated TLR9 activation include promoting cellular proliferation, differentiation into antibody-secreting cells, up-regulating molecules involved in immune cellular interactions and increasing cytokine secretion [8, 9].

A aberrant TLR9 activation has been implicated in the pathogenesis of SLE [10–12], an autoimmune disease characterized by pathogenic autoantibodies to nuclear components [13]. In animal models, activation of autoreactive B cells was mediated by immune complexes.
containing mammalian DNA, through synergistic engagement of the B-cell receptor (BcR) and TLR9 [14, 15]. The B-cell stimulatory cytokine, B-cell activating factor (BAFF), has been implicated in this process [16–20]. In murine B cells, BAFF, BAFF-R and the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) were up-regulated after stimulation with CpG-ODN [21–24]. However, much less is known about the functional cross-talk between TLR9 and BAFF in human B cells. The aim of this work was to investigate the effect of CpG-ODN treatment of normal human B cells on the expression of BAFF and its receptors BAFF-R, TACI and BCMA.

Materials and methods
Isolation of normal human B cells
Normal human B cells were isolated by CD19 microbead separation from peripheral blood mononuclear cells (PBMCs), obtained from healthy adult donors after informed consent. The study was approved by the University of Leicester Research Ethics Committee [Committee for Research Ethics concerning Human Subjects (non-NHS)] and informed consent was obtained. Individual experiments were carried out using B cells from a single donor, and each experiment was carried out on multiple occasions using B cells from different donors. PBMCs were isolated from heparinized blood using Lymphoprep (Axis Shield Diagnostics, UK). Positive selection of the B-cell population was performed by magnetic cell sorting (MACS, Miltenyi, UK) using CD19 microbeads, according to the manufacturer’s protocol. Following separation, the purity of the B-cell population was routinely greater than 95% of lymphocytes.

Cell cultures and CpG-ODN stimulation
Normal human B cells (1 × 10^6 cells/ml) were cultured in RPMI-1640 medium supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin and maintained at 5% CO2 in a humid environment at 37°C. The Class B CpG-ODN, CpG-2006 (TCGTCCATTGTTGGTTTTGTCGTT; InvivoGen, UK), was used as the TLR9 agonist. Preliminary experiments showed that 3 μg/ml CpG-2006 represented the optimal activating dose for human B cells [25], based on the up-regulation of surface markers (CD54, CD86, MHC II) by CpG-ODN.

Immunofluorescent staining and flow cytometry
Cells were stained with the following mouse anti-human monoclonal antibodies: anti-CD86-PE, anti-CD54-PE, anti-MHCII-FITC (Beckman Coulter, UK), anti-BAFF-PE (R&D Systems, UK), anti-BAFF-R-FITC (Santa Cruz Biotechnology, USA), anti-TACI-PE (R&D Systems, UK), anti-BCMA-PE (Enzo Life Sciences, UK) or with relevant isotype control antibodies (Beckman Coulter, UK). Staining for membrane-bound BAFF was carried out using the rat anti-human mAb, Buffy1-FITC (Enzo Life Sciences, UK) or a rat isotype control mAb. After staining, the cells were fixed using 2% cold paraformaldehyde in PBS (pH 7.2), before flow cytometric analysis. For TLR9 staining, the cells were fixed and permeabilized with Perm/Wash buffer (Becton Dickinson, UK), before staining with rat anti-hTLR9-PE (eBioscience, UK). For intracellular BAFF staining, 1 μg/ml of protein transport inhibitor GolgiPlug (Becton Dickinson, UK) containing Brefeldin-A was added for the last 6 h of culture. The cells were fixed and permeabilized as above, prior to staining with mouse anti-hBAFF-PE (R&D Systems, UK) or an isotype control mAb. Flow cytometric analysis was carried out using a FACSCalibur flow cytometer and CellQuestPro software (Becton-Dickinson, UK).

BAFF-specific semi-quantitative PCR
Extraction of total RNA was performed using RNeasy kit (Qiagen, UK), and first-strand cDNA synthesis was carried out using high-capacity cDNA reverse transcription kit (Invitrogen, UK), according to the manufacturer’s instructions. Semi-quantitative PCR was performed using the LightCycler system (Roche Diagnostics, Mannheim). Primer sequences for BAFF-specific PCR were FW: 5’-A GCTGTGTCGCGCGGGAAGTA-3’; RW: 5’-TCACGTCTGCA CTACAGTGGCAAGC-3’. The reactions were prepared using Quantitect SYBR-Green PCR kit (Qiagen, UK), according to the manufacturer’s protocol. The PCR were run for 45 cycles, after optimization of cycling conditions, and the amount of specific PCR product was estimated by the second derivative maximum method. Data were normalized against 18 s rRNA (Quantitect Primer assay; Qiagen, UK) and are presented as arbitrary units.

ELISA
Levels of soluble BAFF were measured in culture supernatants using Quantikine human BAFF/BLYS/TNFSF13B Immunoassays (R&D Systems, UK), according to the manufacturer’s protocols. Culture supernatants were harvested from CpG-2006 (3 μg/ml) stimulated B cells at 24, 48 and 72 h. As a positive control for BAFF secretion, isolated B cells were stimulated with PMA and ionomycin (25 and 250 ng/ml, respectively; Sigma-Aldrich, UK) for 24 h prior to harvesting of culture supernatants. Samples were applied in triplicate wells. The plates were read at 450 nm, with correction at 570 nm, using a NOVOstar plate reader (BMG Labtech, UK).

Cellular proliferation assays
Isolated B cells (2 × 10^5 cells in 200 μl) were treated with 3 μg/ml CpG-ODN, or left untreated, in triplicate wells in round-bottomed 96-well microplates. The cells were pulsed with 1 μCi/well of methyl [3H]thymidine for the final 18 h, before harvesting onto filter mats and counting using a liquid scintillation β-counter (1450 Microbeta Plus; Perkin Elmer, UK). The results are expressed as counts per minute.

For sensitization of CpG-ODN-treated B cells to exogenous BAFF, isolated B cells were treated with 3 μg/ml CpG-2006, or left untreated, for 3 days. Both resting and CpG-ODN-treated cells were then washed and
cultured with 0, 1, 10 or 100 ng/ml of recombinant human BAFF (R&D Systems, UK) for another 3 days, followed by pulsing with [3H]thymidine and estimating cellular proliferation as above. Treatment of cells with anti-IgM (10 µg/ml; Sigma-Aldrich, UK) was used as a positive proliferation control. For blocking studies, the cells were incubated with 50 µg/ml anti-TACI or anti-BCMA blocking monoclonal antibodies (R&D Systems, UK), 10 µg/ml anti-BAFF-R polyclonal antibody (R&D Systems, UK) or relevant control antibodies for 1 h before the addition of exogenous BAFF.

The co-stimulatory activity of surface-bound BAFF was estimated essentially as described by Schneider et al. [26]. Isolated B cells (1 x 10^6 cells/ml) were either left untreated or were treated with 3 µg/ml CpG-2006 for 3 days. Both resting and CpG-ODN-treated cells were then washed and fixed with 1% paraformaldehyde/PBS for 5 min at RT and washed extensively. 2 x 10^5 fixed cells were mixed with 1 x 10^6 freshly isolated B cells in the presence or absence of 5 µg/ml anti-IgM (Sigma-Aldrich, UK) in triplicate wells. The cultures were incubated for another 3 days before pulsing with [3H]thymidine as above. For neutralization experiments, the fixed, CpG-2006-treated cells were incubated for 1 h with 20 µg/ml of either anti-BAFF monoclonal antibody or the control antibody (R&D Systems, UK), and the antibodies were maintained in the cell co-cultures with freshly isolated responder B cells.

Statistical analysis
Statistical analysis was carried out using Prism 5 software (GraphPad, USA). We used paired t-tests for comparison of means of CpG-ODN-treated vs CpG-ODN-untreated groups in matched samples. For all other analyses, analysis of variance (ANOVA) was used. Asterisks in the figures indicate P values, as follows: *P<0.05, **P<0.01, ***P<0.001.

Results
Expression of TLR9 in human B cells and response to CpG-2006
We confirmed the expression of TLR9 in isolated human B cells by monoclonal antibody staining and flow cytometry. TLR9 protein was expressed by virtually all B cells, and the level of expression increased significantly following treatment of the cells with CpG-2006 (Fig. 1A). CpG-2006 treatment of the B cells also resulted in a significant increase in the level of cell surface expression of CD54, CD86 and MHC class II and an increase in B-cell proliferation (Fig. 1B–E). These data confirm previous reports that B cells express TLR9 and respond to CpG-ODN.

CpG-2006 induces membrane-bound BAFF expression by human B cells, but does not induce BAFF secretion
We next investigated the effect of CpG-2006 treatment of isolated human B cells on the expression and secretion of BAFF. CpG-2006 treatment of isolated human B cells resulted in a significant up-regulation of BAFF mRNA after 48 h (Fig. 2A). To investigate whether CpG-2006 stimulated BAFF secretion by isolated human B cells, ELISAs were performed on cell culture supernatants from B cells cultured in the presence or absence of CpG-2006 for 24, 48 and 72 h. PMA and ionomycin induced secretion of BAFF, confirming that human B cells can secrete BAFF under appropriate conditions (Fig. 2B). In contrast, CpG-2006 did not augment BAFF...
secretion over 24, 48 and 72 h of stimulation. However, using intracellular staining and flow cytometry, we observed a consistent increase in the level of BAFF protein expressed in B cells treated with CpG-2006 (Fig. 2C). BAFF is produced as a type II transmembrane protein, which is subsequently cleaved at the cell surface to release its soluble form [26, 27]. We therefore stained the cells for surface expression of BAFF and observed a similar increase in surface expression of BAFF on B cells following their treatment with CpG-2006 (Fig. 2D). To confirm that this represented the membrane-bound form of BAFF, we stained the cells with the monoclonal antibody Buffy-1, which recognizes an epitope that is expressed on the stalk region of membrane-bound BAFF, but is not expressed by the soluble form [28]. CpG-2006 treatment of B cells was associated with an increase in the

**Fig. 2** CpG-2006 does not induce BAFF secretion in isolated human B cells, but does induce expression of membrane-bound BAFF.

(A) Levels of BAFF mRNA, as detected by semi-quantitative PCR, in isolated human B cells. The left panel shows BAFF mRNA in B cells treated with 3 μg/ml CpG-2006 for 24 or 48 h (triplicate values from a single individual; ANOVA); the right panel shows BAFF mRNA levels after 48 h treatment with CpG-2006 (cumulative data from four independent experiments; t-test). (B) CpG-2006 does not induce BAFF secretion by isolated human B cells. Cells were treated for 24, 48 or 72 h with CpG-2006 (3 μg/ml), or left untreated, before soluble BAFF was measured in the culture supernatants by commercial ELISA. B cells treated with PMA and ionomycin for 24 h were used as a positive control (cumulative data derived from four separate donors; ANOVA). (C) Intracellular expression of BAFF protein in isolated human B cells treated with 3 μg/ml CpG-2006 for 72 h, or left untreated.

(continued)
expression of the Buffy-1 epitope (Fig. 2E), indicating expression of the membrane-bound form of BAFF on human B cells. Our data show that TLR9 activation of human B cells induced up-regulation of the membrane-bound form of BAFF but did not induce secretion of soluble BAFF.

CpG-ODN induces expression of TACI and BCMA, but not BAFF-R, on human B cells

The effects of BAFF on B cells are mediated through its interaction with cellular receptors BAFF-R, TACI and BCMA. We therefore examined the expression of these receptors on resting B cells and after stimulation with CpG-2006. Almost all resting B cells expressed BAFF-R, but no increase in BAFF-R expression was observed following 24, 48 or 72 h of CpG-2006 treatment (Fig. 3A). TACI was weakly expressed by resting B cells, and CpG-2006 treatment resulted in a significant upregulation of TACI expression, which was maximal following 72 h of CpG-2006 treatment (Fig. 3B). Dual staining with anti-TACI and Buffy-1 mAbs indicated that both proteins were co-induced in CpG-ODN-treated cells (Fig. 3C). BCMA was weakly expressed by resting B cells. There was no increase in expression of BCMA following 24 or 48 h of treatment with CpG-2006, but a weak up-regulation was seen at 72 h (Fig. 3D).

CpG-ODN treatment of B cells sensitizes them to exogenous BAFF

The induction of TACI expression on a significant proportion of B cells suggested that TLR9 activation might render the cells more sensitive to the effects of exogenous BAFF. To test this, isolated human B cells were pretreated with CpG-2006 for 3 days, before being washed and treated with exogenous BAFF for another 3 days, and the effects on cellular proliferation were measured by [3H]thymidine incorporation. As shown in Fig. 4A, exogenous BAFF (up to 100 ng/ml) alone had no effect on the proliferation of B cells. CpG-2006 (3 µg/ml), on its own, induced a relatively weak increase in cellular proliferation. In contrast, the addition of exogenous BAFF to CpG-2006-pretreated B cells resulted in a dose-dependent increase in the cellular proliferation of the cells, to levels similar to those induced by BcR ligation (anti-IgM; Fig. 4A). This effect was not inhibited by addition of monoclonal antibodies to TACI or to BCMA, even at high concentrations (50 µg/ml; Fig. 4B and D), but was significantly inhibited by an anti-BAFF-R antibody (goat anti-human BAFF-R).
polyclonal antibody; 10 μg/ml; Fig. 4C), suggesting that the effect of exogenous BAFF on B-cell proliferation was mediated, at least in part, through BAFF-R.

CpG-2006-induced membrane-bound BAFF co-stimulates anti-IgM-mediated B-cell proliferation

Finally, we sought to identify whether CpG-2006-induced membrane-bound BAFF played a direct role in B-cell activation. Isolated B cells were either treated with CpG-2006, or left untreated, before being fixed and added to cultures of (unfixed) B cells in the presence or absence of anti-IgM. In the absence of anti-IgM, co-culture of fixed resting or fixed CpG-stimulated B cells with resting (unfixed) B cells did not induce increased cellular proliferation of the unfixed B cells (Fig. 5A), indicating that membrane-bound BAFF did not directly induce resting B cells to proliferate. However, addition of anti-IgM to the co-cultures resulted in an increase in B-cell proliferation.

Fig. 3 CpG-ODN up-regulates TACI and BCMA, but not BAFF-R on human B cells.

(A) Expression of BAFF-R on isolated human B cells treated with 3 μg/ml CpG-2006 for 24, 48 or 72 h, or left untreated. (B) Expression of TACI on isolated human B cells treated with 3 μg/ml CpG-2006 for 24, 48 or 72 h, or left untreated.

(continued)
proliferation that was significantly greater when the fixed B cells had been pretreated with CpG-2006 than when fixed, untreated B cells were added (Fig. 5A). This effect was completely blocked by the addition of an anti-BAFF blocking mAb (Fig. 5B), indicating that membrane-bound BAFF on CpG-2006-treated B cells co-stimulated B-cell proliferation in the presence of anti-IgM.

**Discussion**

Activation of B cells via TLR9 promotes cellular proliferation and differentiation, up-regulates surface molecules involved in immune cellular interactions and increases cytokine secretion [8, 9]. Relatively little is known, however, about the effects of TLR9 activation on the induction of BAFF and its receptors in human B cells, in spite of evidence of a role of TLR9 and BAFF in the pathogenesis of SLE [16, 17, 29–33]. In this article, we show for the first time that CpG-ODN treatment of isolated normal human B cells induced expression of biologically active membrane-bound BAFF, but did not induce BAFF secretion. TLR9 activation also resulted in up-regulation of TACI and BCMA, but not of BAFF-R, and was associated with an increased susceptibility of the cells to proliferate in response to both soluble and membrane-bound BAFF. These findings provide novel insights into the role of TLR9 in human B cells and may be relevant to its role in the pathogenesis of SLE.
In keeping with previous reports [4, 5, 7], we show that isolated human peripheral blood B cells expressed TLR9 and responded to CpG-2006 stimulation by up-regulation of CD54, CD86 and MHC class II and induction of cellular proliferation. We also demonstrated that CpG-2006 treatment was associated with up-regulation of BAFF mRNA and protein expression in human B cells. However, while we saw an increase in the expression of the membrane-bound form of BAFF at the B-cell surface, we did not detect any increase in secreted BAFF in the cell culture supernatants. The expression of the membrane-bound form of BAFF was confirmed by staining the cells with the mAb Buffy-1, which recognizes an epitope expressed in the stalk segment of the membrane-bound form of BAFF, but not in the secreted form [28]. The absence of secreted BAFF following CpG-ODN treatment cannot be explained by an inability of B cells to secrete BAFF per se, as the combination of PMA and ionomycin treatment resulted in BAFF secretion by normal human B cells.

CpG-ODN had no effect on the expression of BAFF-R, but it induced a significant up-regulation of TACI and BCMA, on human B cells. These results indicate a potential difference in the response of human and murine B cells to CpG-ODN, as CpG-ODN induced increased BAFF-R as well as TACI expression on murine splenic B cells [23]. The up-regulation of TACI expression on a significant proportion of B cells following treatment with CpG-2006.
Fig. 5 CpG-ODN-treated B cells co-stimulate the proliferation of anti-IgM-treated B cells.

(A) CpG-ODN-induced membrane-bound BAFF co-stimulates B-cell proliferation with anti-IgM. Isolated human B cells were treated with 3 µg/ml CpG-2006 for 3 days, or left untreated, before being fixed with 1% paraformaldehyde and being washed extensively. The fixed cells were then co-cultured with freshly isolated B cells for 3 days in the presence or absence of anti-IgM antibody (5 µg/ml) (cumulative data from three independent experiments). FR: addition of fixed resting cells to the cultures; FS: addition of fixed CpG-treated cells; untreated cells: responder cells without anti-IgM; anti-IgM: responder cells plus anti-IgM at 5 µg/ml; +20 anti-BAFF: the addition of 20 µg/ml anti-BAFF blocking monoclonal antibody to the cultures; and +20 anti-IgG: the addition of 20 µg/ml IgG isotype control antibody to the cultures (analysis by ANOVA).

(B) The fixed cells were incubated for 1h with 20 µg/ml of anti-BAFF monoclonal antibody, or the control antibody (R&D Systems, UK), prior to adding to the responder B cells; anti-BAFF (or control antibody) was maintained throughout the culture. (Data presented are representative of four independent experiments.) FR: addition of fixed resting cells to the cultures; FS: addition of fixed CpG-treated cells; untreated cells: responder cells without anti-IgM; anti-IgM: responder cells plus anti-IgM at 5 µg/ml; +20 anti-BAFF: the addition of 20 µg/ml anti-BAFF blocking monoclonal antibody to the cultures; and +20 anti-IgG: the addition of 20 µg/ml IgG isotype control antibody to the cultures (analysis by ANOVA).

suggested that TLR9 activation of human B cells might render them more sensitive to the effects of BAFF and/or APRIL. This hypothesis is supported by animal studies, in which CpG-ODN sensitized murine B cells to BAFF-mediated immunoglobulin secretion, through the up-regulation of TACI [23]. We therefore investigated the response of isolated human B cells to exogenous BAFF, in the presence or absence of pretreatment of the cells with CpG-2006. Resting B cells showed no increase in proliferation following addition of exogenous BAFF to the cultures. CpG-2006 on its own induced a modest increase in cellular proliferation, which was significantly enhanced, in a dose-dependent manner, by the presence of exogenous BAFF, to levels that were equivalent to, or greater than, that induced by B-cell receptor signalling induced by anti-IgM antibody. This effect appeared to be mediated through BAFF-R, as blocking antibodies to BAFF-R, but not to TACI or BCMA, significantly inhibited the response to exogenous BAFF. This result could be explained by the abilities of BAFF-R and TACI to differentiate between different oligomeric forms of BAFF, with BAFF-R signalling being activated by both soluble BAFF trimer and higher oligomeric forms, while TACI signalling was only activated by higher order BAFF oligomers [34]. Alternatively, as our study was based on the effects of receptor blocking on B-cell proliferation, this may have been due to the different functions of the receptors [reviewed in 35], with BAFF-R primarily promoting B-cell survival and proliferation, while TACI is involved in antibody production and class switch recombination. We did not see complete inhibition of proliferation in response to exogenous BAFF in cells treated with a polyclonal anti-BAFF-R antibody. This could have been due to the concentration of blocking antibody (as higher concentrations of the control antibody were associated with significant inhibition, limiting the concentration of blocking antibody that we were able to use). Alternatively, this may reflect the properties of this polyclonal anti-BAFF-R antibody, which in addition to inhibiting BAFF-induced proliferation also showed a weak direct effect on B-cell proliferation (data not shown), suggesting that it might not be capable of complete inhibition even at optimal concentrations. The increased proliferation of CpG-treated B cells to exogenous BAFF may be of relevance to SLE, as increased levels of BAFF have been reported in the serum of patients with SLE, and correlated with disease activity [18–20]. As we had shown that CpG-ODN treatment of human B cells induced expression of membrane-bound BAFF on B cells, we investigated whether this was capable of inducing proliferation of resting B cells. We showed that fixed B cells that had been treated with CpG-2006 did not directly stimulate resting B cells to proliferate, but synergized with anti-IgM in inducing B-cell proliferation, and this was blocked by addition of an anti-IgM mAb. These results indicate that CpG-ODN-induced membrane-bound BAFF acted as a co-stimulator of BcR-mediated B-cell activation and demonstrate that CpG-induced, membrane-bound BAFF was biologically active. The results are in agreement with the observations of Schneider et al. [26],
who showed that surface expression of the membrane-bound form of BAFF by stably transfected 293 cells induced proliferation of anti-IgM-stimulated murine B cells, and demonstrate that TLR9 and BcR signalling synergize in the activation of human B cells, in a manner similar to that which has been described for autoreactive B cells in animal models of SLE [14, 15].

Several classes of CpG-ODN have been described, with variations in structure, sequence and function [36]. Class B CpG-ODN, as used in this study, are potent stimulators of B cells and have been used widely in studies of B-cell responses to TLR9 agonists, including studies of B-cell responses to TLR9 agonists in SLE [37, 38]. However, we are not aware of data that indicate that class B CpG-ODN are more important or present in higher concentrations than other classes of CpG-ODN in SLE.

Differentiation of resting B cells into effector cells requires multiple signals and must be strictly regulated to avoid aberrant B-cell activation leading to autoantibody production. Data from animal models implicate TLR9 in the production of autoantibodies to nuclear components in the pathogenesis of SLE. In mice, treatment of B cells with the synthetic TLR9 agonist, CpG-ODN, induced up-regulation of BAFF and APRIL, and BAFF-transgenic mice developed a lupus-like syndrome, which was independent of T cells but dependent on the key TLR signaling regulator, MyD88 [16]. This has led to a model of the pathogenesis of SLE in which apoptotic particles and immune complexes containing nucleic acids activate pDCs and B cells through TLR9 and TLR7, leading to increased secretion of BAFF and APRIL, and up-regulation of BAFF receptors, stimulating further inflammatory cytokine production and autoantibody production [39, 40]. This model is supported by studies in patients with SLE, which showed that patients with active SLE had elevated levels of circulating DNA that was hypomethylated and relatively rich in CpG motifs [41, 42], and had higher levels of TLR9 expression on B cells than controls or patients with inactive disease [29, 30]. Levels of BAFF were elevated in SLE patients’ sera and correlated with levels of dsDNA autoantibodies and/or clinical disease activity [17–20]. B cells from patients with active SLE expressed higher levels of BAFF and APRIL mRNA than B cells from SLE patients with low disease activity or from healthy controls [37], confirming B cells as a possible source of these cytokines. However, while B cells from patients with active SLE expressed BAFF and APRIL mRNA, the levels of expression were lower than was seen in the non-B-cell fraction of PBMC from either healthy individuals or SLE patients [37]. In this study, we have focused on the role of BAFF. Although levels of APRIL are also elevated in patients with SLE, these correlated poorly with disease activity [43, 44]. In addition, unlike BAFF transgenic mice, APRIL transgenic mice exhibited a normal B-cell compartment, without evidence of hyperplasia or autoimmunity [45]. These, and other studies, suggest that the role of APRIL in SLE pathogenesis remains unclear.

Our data suggest that B cells are unlikely to be a major source of soluble BAFF in the response to TLR9 agonists, as we did not see any significant secretion of BAFF into the cell culture supernatant. However, TLR9 stimulation of B cells might contribute to the pathogenesis of SLE in other ways (Fig. 6). First, our data indicate that TLR9 activation renders B cells more sensitive to the effects of BAFF secreted by other cell types. Second, the increase in membrane-bound BAFF expressed on TLR9-activated B cells may act as a co-stimulator of autoreactive B cells, enhancing their proliferation, differentiation and autoantibody production. Our data extend our understanding of the role of TLR9 activation on human B cells and provide new insights into the possible role of TLR9 in the pathogenesis of autoimmune diseases such as SLE.

Fig. 6 Schematic representation of the functional cross-talk between TLR9 and BAFF on human B-cell activation.
References


44 Morel J, Roubille C, Planellas L et al. Serum levels of tumor necrosis factor family members a proliferation-inducing ligand (APRIL) and B lymphocyte stimulator (BLyS) are inversely correlated in SLE. Ann Rheum Dis 2009;68:997–102.