

IRF3-dependent Type I Interferon Response in B Cells Regulates CpG-mediated Antibody Production*

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Hypomethylated CpG oligonucleotides (CpG) are not only potent adjuvants for enhancing adaptive immune responses but may also play a critical role in the development of autoimmune diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). Here we provide evidence that, in addition to dendritic cells, murine B lymphocytes also exhibit a type I IFN response to CpG-B. Unlike dendritic cells, B cell-mediated type I IFN induction depended on the transcription factor IRF3, but similar to dendritic cells this pathway was independent of the IRF3 kinase TBK1. Utilizing type I IFN receptor-deficient mice, we were able to demonstrate that this IFN pathway enhanced Syndecan-1 expression and IgM production and was required for IgG2a production following CpG-B stimulation. Overall, our findings identify a unique IFN pathway in B cells that may play a central role in mediating B cell biology in response to CpG, potentially implicating this pathway in autoantibody production and the pathogenesis of certain autoimmune diseases.

CpG oligonucleotides are adjuvants that potentially enhance Th1 responses with the production of antigen-specific IgG2a, while inhibiting IgE synthesis when injected into mice (1–4). Endogenous and bacterial CpG motifs are also emerging as likely inducers of autoantibody production in diseases such as Systemic Lupus Erythematosus (SLE)⁴ and Rheumatoid Arthritis (RA) (5–10). Mice injected with CpG in a murine model of SLE have significantly elevated anti-DNA antibodies (11). Furthermore, rheumatoid factor-producing B cells are activated by CpG motifs in endogenous DNA when it forms immune complexes with autologous IgG2a in the circulation of autoimmune

mice (12, 13). The ability of CpG to directly activate B cells has been well-characterized as it is one of the most potent B cell mitogens known. CpG stimulates plasma cell differentiation of naïve and memory B cells and can synergize with B cell receptor cross-linking to dramatically enhance B cell proliferation (14, 15). CpG can also directly induce the production of IgG2a, a predominant isotype of autoantibodies in murine models of SLE, without T-cell help (16–18). Nevertheless, the signaling pathways responsible for CpG-mediated IgG2a production are not yet fully understood.

The signaling pathways activated by CpG and other pathogen-associated molecular patterns (PAMPs) have been the focus of extensive study in recent years. CpG motifs present in microbial genomes as well as in host chromatin immune complexes are recognized by Toll-like receptor 9 (TLR9) (19), while lipopolysaccharide (LPS) from Gram-negative bacteria is recognized by TLR4 (20, 21). In bone marrow-derived macrophages (BMMs), both CpG and LPS are able to induce inflammatory cytokine production through a signal transduction pathway that is dependent on MyD88 (22, 23), while only LPS can activate a type I IFN response (24). On the other hand, CpG can induce a strong type I IFN response in dendritic cells, especially in a small subset of dendritic cells known as plasmacytoid dendritic cells (pDCs). LPS activates type I IFN pathway through a MyD88-independent cascade involving TRIF, TBK1, and the transcription factor IRF3, while TLR9 ligation in pDCs induces type I IFN production in a manner that depends on both MyD88 and IRF7 but does not require IRF3 (25). Once produced, type I IFNs play an important role in both innate and adaptive immune responses (26–28). In addition to their beneficial role during viral infections, type I IFNs have been suggested to play a central role in the development of SLE in humans and in murine models (29, 30). Indeed, type I IFN therapy precipitates autoantibody production and SLE-like symptoms in some individuals, suggesting that these IFNs may contribute to the activation of autoreactive B cells (31). In this report we show that B cells exhibit a type I IFN response to CpG in an IRF3-dependent manner, unlike dendritic cells. This type I interferon response enhanced Syndecan-1 expression, IgM production, and was required for CpG-mediated IgG2a production.

EXPERIMENTAL PROCEDURES

Mice—57BL/6 (Jackson Laboratories, Bar Harbor, ME) mice aged 6–12 weeks were used as wild-type controls in all experiments, unless noted. *MyD88*^{−/−} and *Ir3*^{−/−} mouse lines were

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⁴ The abbreviations used are: SLE, Systemic Lupus Erythematosus; IFN, interferon; CpG, unmethylated or hypomethylated CpG oligonucleotides; RA, Rheumatoid Arthritis; DCs, bone marrow-derived dendritic cells; pDCs, plasmacytoid dendritic cells; BMMs, bone marrow-derived macrophages; ELISA, enzyme-linked immunosorbent assay.

on a C57BL/6 background. *Tnfr*^{-/-}, *Tbk1*^{-/-} mice were described previously (32). To obtain *Ifnar*^{-/-} matched littermate wild-type controls on a C57BL/6 genetic background, A129 mice (B&K Universal Limited, Hull, England) were backcrossed with C57BL/6 (Jackson Laboratories) mice for 5 generations. Cells from F5 C57BL/6 littermate wild-type mice were used as wild-type controls for experiments using cells from F5 C57BL/6 *Ifnar*^{-/-} mice. All mice were maintained and bred under SPF conditions in the UCLA-DLAM mouse facility and experiments were conducted within the parameters of our approved protocol (Los Angeles, CA).

Cell Cultures—To obtain highly pure naïve B cells, total splenocytes were stained with a biotin-conjugated anti-CD43 antibody (PharMingen) followed by streptavidin-conjugated magnetic microbeads (Miltenyi Biotec, Auburn, CA) and passed through a negative selection magnetic sorting column (Miltenyi Biotec). Unbound cells were collected as purified resting B cells, which were over 98% pure B220⁺, CD11C⁻ B cells. B cells were cultured in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum, 50 μ M β -mercaptoethanol, and 1% penicillin/streptomycin (Invitrogen) at 37 °C under 10% CO₂. Murine bone marrow-derived macrophages (BMMs) were differentiated from adherent bone marrow cells. Briefly, bone marrow cells were isolated and maintained in macrophage differentiating media (1 \times Dulbecco's modified Eagle's medium (Mediatech Inc., Herndon VA), 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μ g/ml), and 30% L929 conditioned medium) for 7 days. Murine bone marrow-derived dendritic cells (mDCs) were differentiated from non-adherent bone marrow cells. Briefly, bone marrow cells were isolated and maintained in dendritic cell differentiating media (1 \times RPMI (Mediatech Inc., Herndon VA), 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μ g/ml), and GM-CSF (10 ng/ml)) for 6 days, during which time the media was changed every 2 days. To obtain Flt-3 DCs (pDC), bone marrow cells were isolated and differentiated in RPMI (Mediatech Inc., Herndon VA), 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μ g/ml), 2-mercaptoethanol, and 100 ng/ml murine Flt3-ligand (R&D Systems, Minneapolis, MN) for 7 days prior to stimulation. Media was replaced 4 days into the 7-day-culture period. Cells were stimulated with lipopolysaccharide (5 μ g/ml) (Sigma) or unmethylated CpG-containing oligonucleotides (75 nM), referred to as CpG-B in the text and figures, (5'-TCCATGACGTTTCCTGACGTT-3') with a phosphorothioate backbone (Invitrogen).

Immunoblotting—Anti-STAT1 antibodies were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Antibodies specific to the phosphorylated forms of STAT1 were obtained from Cell Signaling Technologies (Beverly, MA). Cell lysates were quantified and 25 μ g of cytoplasmic extracts were loaded in each lane and separated by SDS-PAGE. Gels were transferred to Immobilon-P membranes (Millipore Corp., Billerica, MA) and immunoblotted using the antibody manufacturers' recommended instructions.

Quantitative Real-time PCR (Q-PCR)—RNA was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. RNA was quantitated, DNase-treated (Ambion), and 1 μ g of RNA was used to make cDNA templates

using iScript (Bio-Rad) according to the manufacturer's instructions. Q-PCR analyses were done using the iCycler thermocycler (Bio-Rad). Q-PCR was conducted in a final volume of 20 μ l containing: Taq polymerase, 1 \times Taq buffer (Stratagene), 125 μ M dNTP, SYBRTM Green I (Molecular Probes, Eugene, OR), and fluorescein (Bio-Rad), using oligo-dT cDNA as the PCR template. Amplification conditions were: 95 °C (3 min), 40 cycles of 95 °C (20 s), 55 °C (30 s), 72 °C (20 s). Primers were used to amplify specific 80–120-bp fragments corresponding to their respective genes. Primer sequences for *18s*, *Mx1*, *Ip10*, *Ifna4*, and *Ifnb* are the same as those previously published (24, 33, 34). The primer sequence for *IL10* was TCATCGATT-TCTCCCCTGTGA, forward, and GACACCTTGGTCTTG-GAGCTTATT, reverse. *18s* expression measurements were conducted in tandem with the gene of interest. All data are presented as relative expression units after normalization to the average *18s* value to control for loading of total RNA (35). Measurements were conducted in duplicates or triplicates.

ELISA—IgM, IgG2a, and IgE antibodies were detected in culture supernatants by ELISA, under the same conditions as described below for survival and proliferation studies. 96-well plates were coated with 2 μ g/ml rabbit anti-mouse IgM, anti-IgG (H+L), or anti-IgE (Southern Biotechnology) in sodium carbonate buffer, pH 9.6 overnight and blocked with 1% bovine serum albumin (Sigma) for 1 h. After a 1-h incubation with culture supernatants and standards (eBiosciences), plates were washed and further incubated with horse radish peroxidase-conjugated goat anti-mouse IgM, anti-mouse IgG2a, or anti-mouse IgE (Southern Biotechnology). After washing, we quantified the amount of enzyme bound to each well with the OPD peroxidase substrate (Sigma). The absorbance of the colored reaction product was read at 492 nm by a 96-well plate reader (Fisher Scientific). IFN α was assayed using a murine IFN α ELISA kit from PBL Biomedical Laboratories (Piscataway, NJ), while IL-6 and IL-12 were assayed using mouse ELISA kits from eBioscience (San Diego, CA) according to the manufacturer's instructions.

Flow Cytometry—Cells were cultured at 10⁶ cells/ml in 96-well plates. For Syndecan-1 staining, cells were stained with 1:200 dilution of PE-conjugated anti-Syndecan-1 antibody (PharMingen), washed twice and analyzed on FACScan (Becton Dickinson). Analysis was conducted using CellQuest software (BD Biosciences).

Proliferation and Cell Viability—For proliferation studies, B cells were stimulated as indicated at 10⁶ cells/ml in 96-well plates. 18–20 h later, 0.5 μ Ci of [³H]thymidine was added to the cultures and incubated for another 16 h before harvest. Cultures were collected on a 96-well filtermat with an automated harvester. Activity was assessed using a scintillation counter, and counts per minutes (CPM) were reported. To assess cell viability, cells were stimulated as mentioned above and stained with propidium iodide (PI). The PI-negative population was determined by FACS and reported as percent viable cells.

RESULTS

CpG Induces STAT1 Phosphorylation and Type I IFN-regulated Genes in Both B Cells and DCs—Although several cell types express TLR9, only pDCs are known to display a potent

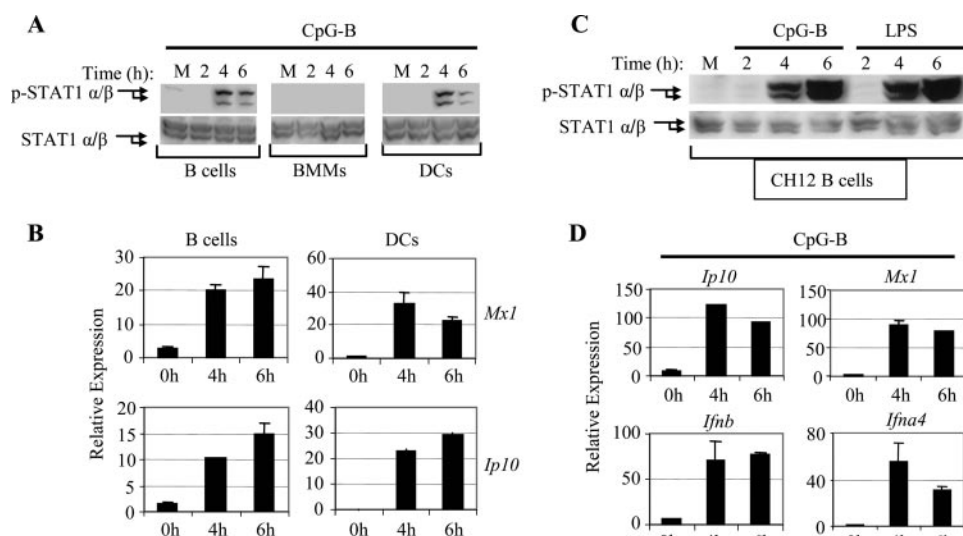


FIGURE 1. CpG induces STAT1 phosphorylation and IFN target gene induction in B cells and DCs. A and B, purified B cells, BMMs, and GM-CSF-derived DCs from C57Bl/6 mice were stimulated with CpG-B (75 nM) for the indicated timepoints. A, protein extracts were analyzed by SDS-PAGE immunoblotting using antibodies specific for phosphorylated-STAT1 (Y701) or total STAT1 as a loading control. B, RNA was isolated and analyzed by Q-PCR for expression of indicated genes. C and D, CH12 B cells were stimulated with CpG-B or LPS (5 μ g/ml) for the indicated timepoints. Protein extracts and RNA were isolated and analyzed as in A and B, respectively. Q-PCR results are expressed in relative expression units and have been normalized to 18s RNA levels. Data represent at least two independent experiments. Error bars indicate \pm SD among duplicates.

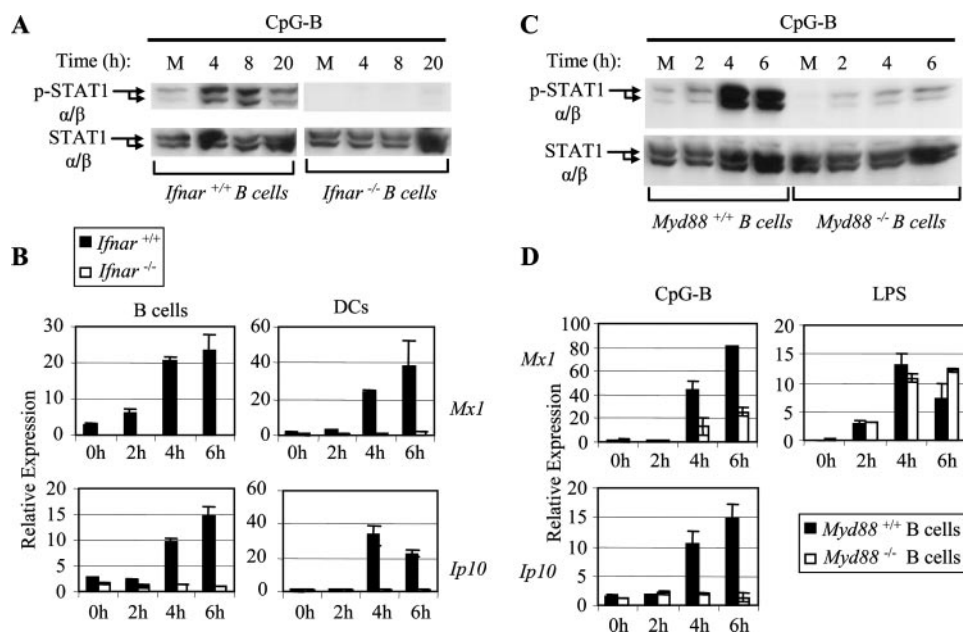


FIGURE 2. CpG-induced STAT1 phosphorylation and IFN target gene induction require IFNAR and MyD88. A and B, *Ifnar*^{+/+} and *Ifnar*^{-/-} purified B cells or GM-CSF-derived DCs were stimulated with CpG-B for the indicated timepoints. Protein extracts and RNA were isolated and analyzed as in Fig. 1. C and D, *Myd88*^{+/+} or *Myd88*^{-/-} purified B cells were stimulated with CpG-B or LPS for the indicated timepoints. Protein extracts and RNA were isolated and analyzed as above. Data represent at least two independent experiments. Error bars indicate \pm SD among duplicates.

type I IFN response to CpG (36). Two different CpG-containing DNA oligonucleotides, CpG-A and CpG-B, have been shown to induce Type I IFNs in pDCs (37). Although CpG-A induces higher levels of type I IFNs, only CpG-B serves as a potent activator of B cells (37, 38).

It has been previously reported that stimulation of total splenocytes with CpG-B induces strong STAT1 phosphorylation (9). STAT1 can be activated by both IFN γ through the

IFN γ receptor or IFN α/β through the type I IFN receptor (IFNAR) (39). Therefore, in splenocytes this response could be mediated by various cell types activated by CpG-B. To compare different cell types, we stimulated purified primary B cells, BMMs, and bone marrow-derived cells (DCs) with CpG-B. As shown in Fig. 1A, both B cells and DCs induced strong STAT1 phosphorylation when incubated with CpG-B, while BMMs did not. Furthermore, both B cells and DCs, but not BMMs, increased expression of the IFN target genes, *Mx1* and *Ip10*, following CpG-B stimulation (Fig. 1B and data not shown). Although both IFN γ and IFN α/β can activate *Ip10*, induction of *Mx1* is specific to IFN α/β , suggesting that this response is mediated through type I IFNs rather than IFN γ (39). We found it interesting that purified B cells induced this IFN response and concentrated our further studies to understand how this pathway is activated.

Although the possibility that STAT1 phosphorylation and IFN-dependent gene induction results from contaminating cells in our primary cell isolation cannot be completely excluded, we also found that both LPS and CpG-B strongly induced STAT1 phosphorylation in the well-established CH12 B-cell line, which are murine lymphoma-derived IgM⁺ B cells, with similar kinetics as primary B cells (Fig. 1C) (40, 41). In addition, RNA from CpG-B-treated CH12 cells was analyzed and demonstrated a potent induction of *Ip10*, *Mx1*, *Ifna4*, and *Ifnb* mRNAs (Fig. 1D). Thus, CpG-B induces STAT1 phosphorylation and up-regulates type I IFN target genes in both B cells and GM-CSF-derived dendritic cells.

CpG-induced STAT1 Phosphorylation and IFN Target Gene Induction Require IFNAR and MyD88—To definitively demonstrate that STAT1 phosphorylation in primary B cells was dependent on type I IFNs rather than IFN γ signaling, we stimulated *Ifnar*^{+/+} and *Ifnar*^{-/-} B cells with CpG-B. STAT1 phosphorylation and subsequent IFN target gene induction were both abolished in *Ifnar*^{-/-} B cells (Fig. 2, A and B). IFNAR was also required for IFN target gene induction in DCs (Fig. 2B). Thus, B cells, similar to dendritic

cells, induce STAT1 phosphorylation and subsequent IFN target gene induction in a type I IFN-dependent manner in response to CpG-B stimulation.

MyD88 is a critical adaptor molecule for TLR9-mediated type I IFN production in pDCs following CpG stimulation (37). However, recent reports have also shown that B-form DNA can induce type I IFNs in a TLR- and MyD88-independent fashion (42). To determine if CpG-mediated type I IFN response in B cells is MyD88-dependent, wild-type and *Myd88*^{-/-} B cells were stimulated with CpG-B. Fig. 2, C and D demonstrates that both CpG-B-mediated STAT1 phosphorylation and IFN target gene induction were defective in MyD88-deficient B cells. Furthermore, LPS induces type I IFNs through a TRIF-dependent pathway, and as expected, *Mx1* induction in response to LPS

was indeed independent of MyD88 (Fig. 2D) (43). Thus, similar to DCs, B cells rely on MyD88 to activate the type I IFN pathway in response to CpG-B, indicating that this pathway is initiated through a TLR-dependent mechanism.

IRF3 Is Required for CpG-induced IFN Response in B Cells but Not in DCs—Previous studies have shown that IRF3 is required for type I IFN production in response to LPS stimulation, but not required for CpG-A-mediated type I IFN production in pDCs (25, 33). To determine if IRF3 is involved in CpG-B-mediated type I IFN response in B cells, we compared the IFN response in wild-type and *Irif3*^{-/-} cells stimulated with CpG-B. As shown in Fig. 3, A and B, CpG-B-induced STAT1 phosphorylation and IFN target gene induction were defective in *Irif3*^{-/-} B cells. This defect was specific to type I IFN production as *IL10* induction was similar in both *Irif3*^{+/+} and *Irif3*^{-/-} B cells (Fig. 3C). In contrast, the type I IFN response was not significantly affected in both *Irif3*^{-/-} mDCs and pDCs, as demonstrated by similar STAT1 phosphorylation and induction of *Irfnb* and IFN target genes in mDCs, as well as production of IFN α in pDCs (Fig. 4, A–D). As expected, IRF3 was still required for LPS-mediated *Ip10* and *Mx1* induction in both B cells and DCs (Figs. 3B and 4B). These findings provide strong evidence that CpG-B-mediated induction of the type I IFN response is dependent on the transcription factor IRF3 in B cells but not in DCs.

CpG-mediated IFN Response in B Cells Is Independent of TBK1—TLR4 activates IRF3 and induces type IFNs through a mechanism that is completely dependent on TBK1 (32, 44). In contrast, TLR9 induces type I IFNs through the activation of IRF7, and this pathway is independent of TBK1 (45). We therefore wanted to investigate the role of TBK1 in CpG-B-mediated type I IFN response in B cells (32, 44, 45). As *Tbk1*^{-/-} mice are not viable, *Tnfr1*^{-/-}, *Tbk1*^{+/+} and *Tnfr1*^{-/-}, *Tbk1*^{-/-} B cells, and DCs were stimulated with either CpG-B or LPS. Unlike LPS, CpG-B did not require TBK1 for type I IFN-dependent STAT1 phosphorylation in either B cells or DCs (Fig. 5, A and B). Thus, TLR9 in B-cells utilizes a distinct pathway to induce a type I IFN response from that utilized by TLR4 in B cells or TLR9 in DCs.

CpG-mediated Type I IFN Response in B Cells Regulates Survival, IgM-secreting Plasmablast Differentiation and Is Required for IgG2a Production—Both CpG-B as well as exogenous type I IFNs are known to induce B cell differentiation to antibody-secreting plasmablasts (15, 27). To determine if the type I IFN response activated by CpG-B affected B cell differentiation, we stimulated purified primary B cells from wild-type and *Irfnar*^{-/-} mice with CpG-B and monitored B cell survival, proliferation, and antibody production. *Irfnar*^{+/+} and *Irfnar*^{-/-} B cells responded similarly to the CpG-B stimulation as measured by proliferation and ICAM-1

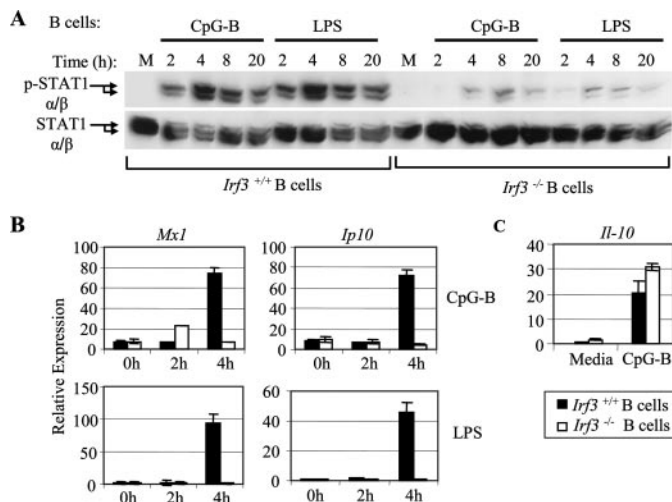


FIGURE 3. IRF3 is required for CpG-induced IFN response in B cells. A–C, *Irif3*^{+/+} or *Irif3*^{-/-} purified B cells were stimulated with CpG or LPS for the indicated timepoints. Protein extracts and RNA were isolated and analyzed as above. Data represent at least two independent experiments. Error bars indicate \pm SD among duplicates.

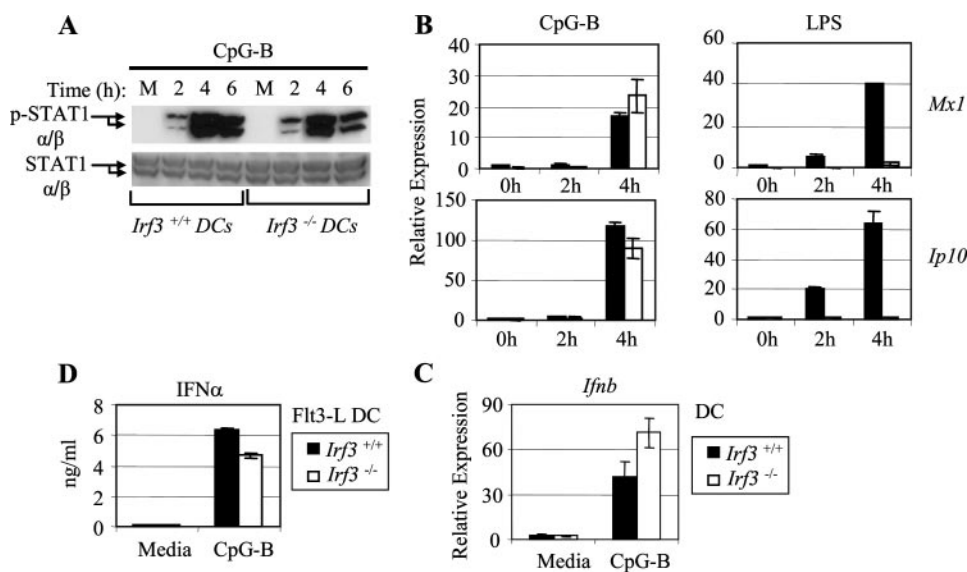


FIGURE 4. IRF3 is dispensable for CpG-induced IFN response in dendritic cells. A–C, *Irif3*^{+/+} or *Irif3*^{-/-} GM-CSF-derived DCs (mDCs) were stimulated with CpG-B or LPS for the indicated timepoints. Protein extracts and RNA were isolated and analyzed as above. D, *Irif3*^{+/+} or *Irif3*^{-/-} Flt3-ligand-derived DCs (pDCs) were stimulated with CpG-B for 24 h. Supernatants from cultures were analyzed for the presence of IFN- α by ELISA. Data represent at least two independent experiments. Error bars indicate \pm SD among duplicates.

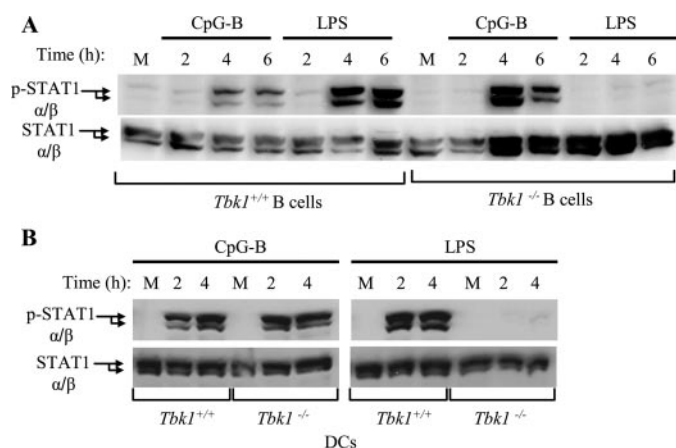


FIGURE 5. CpG-induced type I IFN response is TBK1-independent in B cells and DCs. A and B, *Tnfr1*^{-/-}, *Tbk1*^{+/+}, and *Tnfr1*^{-/-}, *Tbk1*^{-/-}-purified B cells or GM-CSF-derived DCs were stimulated with CpG-B or LPS for the indicated timepoints. Protein extracts were isolated and analyzed as above.

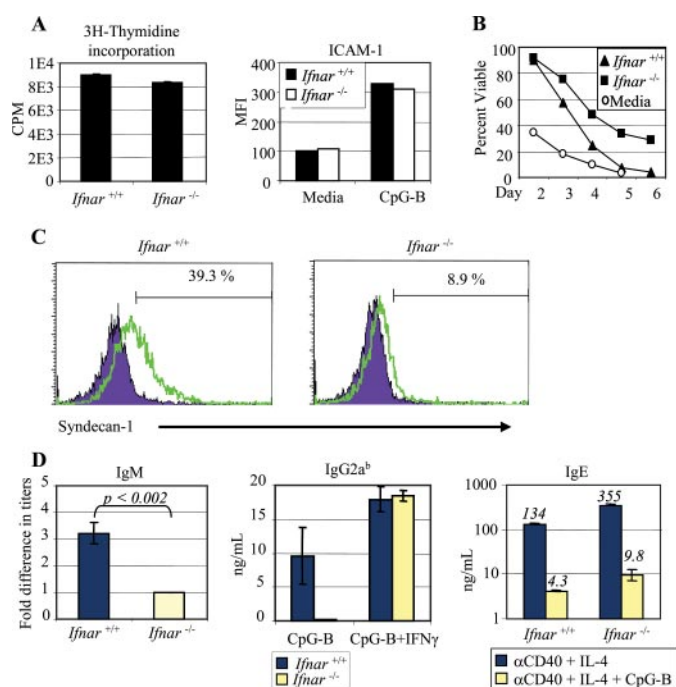


FIGURE 6. CpG-mediated Type I IFN response in B cells regulates survival and antibody production. A, *Ifnar*^{+/+} and *Ifnar*^{-/-}-purified B cells were stimulated with CpG-B. Surface ICAM-1 expression was analyzed by flow cytometry after 24 h of CpG-B stimulation. Proliferation was assessed by [³H]thymidine incorporation. B, *Ifnar*^{+/+} and *Ifnar*^{-/-}-purified B cells were stimulated either with CpG-B or left in media alone. Cells were stained daily with propidium iodide (PI) and analyzed by flow cytometry. The PI-negative population is expressed as percent viable cells. C, After 5 days of stimulation, B cells from indicated genotypes were stained with anti-Syndecan-1 antibody and analyzed for Syndecan-1 expression by flow cytometry. Isotype control for each sample is shown as a filled curve. D, supernatants from 5-day CpG-B-stimulated B cell cultures were assessed for the presence of IgM by ELISA. Supernatants from 6-day cultures were assessed for the presence of IgG2a by ELISA. Supernatants from 9-day cultures were assessed for the presence of IgE by ELISA. Data represent at least two independent experiments. Error bars indicate \pm SD among duplicates.

up-regulation (Fig. 6A). However, *Ifnar*^{-/-} B cells displayed enhanced survival as compared with wild-type cells after several days of incubation with CpG-B (Fig. 6B). These results are consistent with previous reports that type I IFNs induce cell death in B cells (46). We further assessed the functional role of

the type I IFN response in B cells by analyzing IgM production and Syndecan-1, a plasma cell marker, expression after 5 days of stimulation. CpG-B-treated wild-type B cells produced around 3-fold higher levels of IgM and expressed significantly higher levels of Syndecan-1 compared with *Ifnar*^{-/-} B cells (Fig. 6, C and D, left). It is important to note that IgM levels in the supernatants of CpG-B-stimulated wild-type B cells were consistently higher than that of IFNAR-deficient B cells despite the fact that much fewer wild-type cells were viable at this time point (Fig. 6B). These findings suggest that, although not required, the type I IFN response activated by CpG-B in B cells can significantly enhance plasmablast differentiation and IgM production.

CpG-B is known to induce IgG2a production in B cells *in vitro* (47). STAT1 has also been implicated in class switch recombination (CSR) through induction of the transcription factor T-bet (48). Finally, a recent study has shown that IFN- α augmentation of antibody response *in vivo* requires IFNAR on B cells (49). To determine if the type I IFN-dependent response observed in B cells plays a role in CpG-B-mediated IgG2a production, IgG2a levels were assessed in the supernatants of CpG-B-stimulated B cells. Interestingly, a significant defect was observed in IgG2a production in *Ifnar*^{-/-} B cells compared with wild-type B cells (Fig. 6D, middle). This was not due to a general defect in isotype switching as IFN γ rescued IgG2a production in *Ifnar*^{-/-} B cells (Fig. 6D, middle). Again, the defect in IgG2a production was observed despite the fact that *Ifnar*^{-/-} B cells had relatively higher viability compared with wild-type cells (Fig. 6B). Finally, we also found that CpG-B inhibition of IgE and IgG1 production was comparable between *Ifnar*^{+/+} and *Ifnar*^{-/-} B cells (Fig. 6D, right and data not shown). Interestingly, there was a tendency of higher IgE production in *Ifnar*^{-/-} B cells compared with wild-type cells, although the difference did not reach significance (Fig. 6D, right). Overall, it appears that CpG-B-induced type I IFNs in B cells may not play a significant role in the inhibition of Th2 antibody production but are important for the production of the Th1 antibody IgG2a.

DISCUSSION

Hypomethylated CpG motifs induce strong proliferative and survival signals in B cells and are thought to activate autoreactive B cells in autoimmune diseases (12–14). Type I IFNs are also thought to play a critical role in autoimmune diseases (29–31). Previous studies have suggested that the major producers of type I IFNs are pDCs, and the major target of these IFNs contributing to the development of SLE are immature DCs (50). However, recent studies have shown that B cells may serve as targets of type I IFNs during T cell-dependent antibody responses *in vivo* (49, 51). Specifically, mice whose B cells lacked IFNAR failed to produce antigen-specific IgG2a when type I IFNs were used as an adjuvant. Our results now not only show that B cells are direct targets of type I IFNs but also B cells themselves may secrete these interferons following Toll-like receptor activation at levels sufficient to significantly influence B cell biology.

While pDCs are the most potent type I IFN producing cell type, our results provide strong evidence that B cells can also

exhibit a type I IFN response to CpG-B. We clearly demonstrate that STAT1 phosphorylation and IFN-regulated gene induction in B cells was dependent on the IFNAR receptor, indicating that type I IFNs were produced in these cells. Type I IFN protein was not measurable in this system, likely due to the level of detection of the assay not representing physiologic active concentrations. Although we cannot exclude the possibility that contaminating DCs in our B cell isolates are producing the type I IFNs responsible for the observed results, we believe this is not the case for several reasons. First, the purity of our B cell isolates is consistently over 98% B220⁺, CD11c⁻. Second, we observe the same CpG-B-induced IFN response with direct *Ifna/b* gene induction in a pure B cell line. Finally, CpG-B-induced STAT1 phosphorylation and downstream IFN target gene induction in primary B cells were all IRF3-dependent. In contrast, type I IFN production in DCs was IRF3-independent. IRF3 may not be required in DCs because of constitutive expression of IRF7. In addition, IRF7 may also allow for higher levels of type I IFN production in response to TLR9 ligation observed in DCs compared with B cells. In addition, LPS induction of type I IFN response in purified primary B cells is further evidence that B cells are capable of producing type I IFNs. We would also argue that CpG-B-induced type I IFN response is not due to LPS contamination because the CpG-B response is MyD88-dependent and TBK1-independent, while the LPS-induced IFN response in B cells is MyD88-independent and TBK1-dependent. Thus, the CpG-B-induced type I IFN response in B cells is distinct from both the CpG-B-induced response in DCs and the LPS-induced response in B cells in that it depends on both MyD88 and IRF3 but not TBK1.

Our results indicate that CpG-B-mediated IgG2a production in purified naïve B cells *in vitro* is defective in cells that lack the receptor for type I IFNs. It is interesting to note that, while the type I IFN response was required for IgG2a production, the ability of CpG-B to inhibit IgE secretion was unaffected by IFNAR deficiency, although there was a general tendency of higher IgE production in IFNAR-deficient cells. Collectively, these data suggest that type I IFN pathway in B cells may skew the antibody response toward Th1 antibodies. In addition, we noted higher levels of Syndecan-1 expression and IgM production in wild-type compared with IFNAR-deficient B cells stimulated with CpG-B, where *Ifnar*^{-/-} cells displayed survival advantage. These findings suggest that type I IFNs enhance B cell differentiation into short-lived plasmablasts as well as contribute to cell death. Interestingly, B cells from SLE patients also exhibit accelerated cell death *in vitro* (52). Previous studies have also demonstrated a type I IFN "signature" in leukocytes from SLE patients, which may resemble the type I IFN response we observe in CpG-B-stimulated B cells (53). Future studies may therefore help assess the contribution of altered type I IFN production in SLE patients to plasma cell differentiation and accelerated cell death of B lymphocytes. The direct induction of T cell-independent antibody secretion that is augmented by the type I IFN pathway in B cells could also have significant biological implications. Although this pathway could be beneficial in physiological responses, as in viral infections, the production of self-reactive antibodies in the absence of T cell help might also

regulate pathology in autoimmune diseases. Thus, the type I IFN pathway in B cells may be an enticing therapeutic target.

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