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Yi-Kai Chiu, I-Ying Lin, Shin-Tang Su, Kuan-Hsiung Wang, Shii-Yi Yang, Dong-Yan Tsai, Yi-Ting Hsieh and Kuo-I Lin

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Ag-primed B cells that result from an immune response can form either memory B cells or Ab-secreting plasma cells; however, the molecular machinery that controls this cellular fate is poorly understood. In this study, we show that activated B cell factor-1 (ABF-1), which encodes a basic helix-loop-helix transcriptional repressor, participates in this regulation. ABF-1 was prevalently expressed in purified memory B cells and induced by T follicular helper cell–mediated signals. ABF-1 expression declined by the direct repression of B lymphocyte–induced maturation protein-1 during differentiation. Ectopic expression of ABF-1 reduced the formation of Ab-secreting cells in an in vitro differentiation system of human memory B cells. Accordingly, knockdown of ABF-1 potentiates the formation of Ab-secreting cells. A transgenic mouse that expresses inducible ABF-1 in a B cell–specific manner was generated to demonstrate that the formation of germinal center and memory B cells was augmented by induced ABF-1 in an immune response, whereas the Ag-specific plasma cell response was dampened. This effect was associated with the ability of ABF-1 to limit cell proliferation. Together, our results demonstrate that ABF-1 facilitates formation of memory B cells but prevents plasma cell differentiation. The Journal of Immunology, 2014, 193: 2207–2217.

Immunological memory, adapting from a primary immune response to a given Ag, is the central basis of vaccination. In a T cell–dependent immune response, T follicular helper (Tfh) cells that encounter Ag-primed B cells in the secondary lymphoid organs provide cytokines, such as IL-21, and costimulatory interaction including CD40L to support Ag-primed germinal center (GC) B cells. This process instructs the differentiation of either memory B cells or Ab-secreting plasma cells (1, 2). Preceding the differentiation to form memory B or plasma cells, GC B cells undergo somatic hypermutation of Ig variable regions to acquire the enhanced ability to recognize Ags. The molecular bases involved in deciding the specification of GC B cells to differentiate into memory B cells or plasma cells are largely unknown. The gene-regulatory networks controlling the generation of plasma cells have been demonstrated. The induction of B lymphocyte–induced maturation protein-1 (Blimp-1), encoded by PRDM1, in Ag-primed B cells is essential for plasma cell fate (3, 4). IFN regulatory factor 4 is important for the initiation of plasma cell differentiation and Blimp-1 induction (5, 6). Other mechanisms leading to the induction of Blimp-1 during the differentiation plasma cells include the downregulation of mature B or GC B cell genes, Pax5, and Bcl6 (6, 7). Downregulation of Pax5 permits the induction of Xbp-1 that is crucial for the secretion of Ab (8, 9). However, the transcription factors pivotal for the establishment of memory B cell fate are not known.

A previous microarray study showed that Blimp-1 drives plasma cell differentiation by repressing genes that are involved in B cell identity and GC B cell function (10). One of the genes revealed by that study encodes basic helix-loop-helix transcription factor activated B cell factor-1 (ABF-1). Transcription factor E proteins have many important functions during immune cell development/differentiation, especially in lymphocytes (11). There are four E proteins in mammals: E12, E47 (both encoded by E2A), HEB, and E2-2 (11–13). ABF-1, also known as musculin (MSC), was discovered with a yeast two-hybrid screen of a B cell cDNA library using the bHLH region of E2-2 as bait (14). ABF-1 binds to the E-box element to form a homodimer or heterodimer with E12 or E47 (14). The transcriptional activity of E47 is attenuated by the repression domain located in the C-terminal half of ABF-1 (14, 15). ABF-1 is expressed in human lymphoid tissues including lymph nodes, fetal liver, and bone marrow, EBV-transformed lymphoblastoid cell lines, and activated human B cells (14). The physiological function of ABF-1 in human B lymphocytes has not been determined. High expression of ABF-1 and Id2 in classical Hodgkin lymphomas inhibits E2A activity, which causes the loss of expression of B cell–associated genes and upregulation of aberrant genes such as TCFF and CSFIR that are not normally expressed in the B cell lineages (16). The mouse homolog of ABF-1, MyoR (myogenic repressor), or Msc, is predominantly expressed in undifferentiated, proliferating myoblasts and is downregulated during differentiation (17, 18); whether it is involved in B cells is still unknown.

In this study, we sought to pursue the function of ABF-1 in the context of plasma cell differentiation and uncovered the role of
ABF-1 in the promotion of GC and memory B cell fate and the suppression of plasma cell formation.

**Materials and Methods**

**Cell culture and reagents**

Human B cells were purified first with a RosetteSep HLA B cell Enrichment Cocktail (StemCell Technologies) through negative selection followed by incubation with anti-CD27-coupled magnetic beads (Miltenyi Biotec) to isolate memory B (CD27+) cells. Naïve B (CD27-) cells were obtained from the flow-through fraction. Purified human B cells at a density of 1 × 10⁶ cells/ml were stimulated with IL-21 (100 ng/ml; Invitrogen) and anti-CD40 (1 μg/ml; R&D Systems). SKW 6.4 (SKW) human lymphoblastoid cells, 18-81 pre-B cells, 293T cells, and 3T3 cells were maintained as described (10, 19). Stable WI-L2 transfectants were maintained as described (10), and Blimp-1–estrogen ligand-binding domain (ERD) was induced by 5 μM DiCl3 (Sigma–Aldrich) and 3 μM 4-OHT (Sigma–Aldrich) treatment for 10 h. Mouse splenic B cells were purified by positive selection using B220 microbeads (Miltenyi Biotec), and the purified B cells were stimulated with LPS (2 μg/ml), goat anti-mouse F(ab)’2 (10 μg/ml; Jackson Immunolaboratory Researches), anti-CD40 (1 μg/ml; BD Biosciences), and IL-21 (200 ng/ml; eBioscience) at a density of 1 to 2 × 10⁶/ml.

**Plasmids**

Details of plasmid information, including ABF-1 tagged with GFP at the C terminus (ABF-1–GFP), shRNA against ABF-1 and GFP–Blimp-1 expression by lentiviral vector, plasmid (24) are available upon request. The genomic fragment containing –2967 to +318 of ABF-1 was amplified from genomic DNA of primary human peripheral mononuclear cells and was cloned into pGL3 basic vector (Promega) to generate ABF-1–Luc. Details of the construction of various derivatives of ABF-1–Luc that carry mutated sequences by site-directed mutagenesis at the Blimp-1 binding sites are available upon request.

**Generation of EpVp–ABF–1–estrogen receptor transgenic mice and immunization**

To express ABF-1 in B cells in an inducible manner, we generated transgenic (TG) mice with ABF-1 cDNA fused in-frame to the N-terminal region of mouse estrogen receptor (ER) and then subcloned ABF-1–ER cDNA into pEpVp (provided by Dr. Suzanne Cory, Walter and Eliza Hal Institute), in which ABF-1–ER transcription was controlled by the IgM enhancer (Eα) and a V γ promoter (21). The TG mice were produced by Level 1 (Taiwan, and were maintained in C57BL/6 genetic background. Germine transmission of ABF-1–ER by TG founders and the subsequent confirmation of TG passages were conducted by PCR with primers spanning the fusion region of the cDNA sequences of ABF-1 and ER (primer sequences 5′-CTCAGGCCGAGAGTGCAAGC-3′ and 5′-CTTCTTCCCTGGACAGTGA-3′) were normalized as described in a previous report (19). Regions encompassing or adjacent to Blimp-1 binding sites were amplified by QPCR. The genomic fragment containing –2967 to +318 of ABF-1 was amplified from genomic DNA of primary human peripheral mononuclear cells and was cloned into pGL3 basic vector (Promega) to generate ABF-1–Luc. Details of the construction of various derivatives of ABF-1–Luc that carry mutated sequences by site-directed mutagenesis at the Blimp-1 binding sites are available upon request.

**DNA pulldown assay and EMSA**

The DNA pulldown assay was performed essentially as described (23). Recombinant truncated form of Blimp-1 (tBlimp) was expressed and purified according to a previous report (24). The EMSA was performed with a LightShift chemiluminescence EMSA kit (Pierce) (23). Briefly, 2 μg nuclear extract from 293T cells transfected with Blimp-1 expression vector was incubated with 45 fmol biotin-labeled probe in the absence or presence of the indicated excess amount of unlabeled oligonucleotides. In the supershift assay, 0.5 μg mouse anti–Blimp-1 Ab (23) or control mouse IgG (Sigma–Aldrich) was added to the reaction. The oligonucleotides corresponding to various Blimp-1 binding sites and the negative control oligonucleotides are listed in Supplemental Table I.

**Transfection and luciferase reporter assay**

ABF-1–Luc (15 μg) or its various mutants, various amounts of Blimp-1 expression vector (pCMV-Blimp1) or control vector (pCMV-5b), and 0.3 ng Renilla luciferase reporter driven by the thymidine kinase promoter (RL-k) were cotransfected into 18-81 B cells by electroporation at 230 V and 950 μF. After 18 h, cells were lysed and subjected to firefly luciferase and Renilla luciferase assays using the Dual-Luciferase Reporter Asssay kit (Promega). The luminescence was measured by TopCount NXT (PerkinElmer). Fold repression was calculated as described (19).

**Generation of lentivirus and viral transduction**

The procedure for preparing lentiviral vectors was described as previously described (19). Purified memory B cells stimulated with IL-21 plus anti-CD40 for overnight, and SKW cells were transduced with lentiviral vector at the multiplicity of infection of 4–10. The percentage of cells expressing GFP in transduced populations reached >95% in SKW cells 2 d after transduction. The transduced, GFP+, human B blood cells were sorted by FACSAria (BD Biosciences).

**ELISA and ELISPOT assays**

Cell-culture supernatants were harvested for ELISA to determine the amount of IgM or IgG as described (9). ELISPOT assay for detecting IgG-secreting cells essentially followed a previously reported procedure (9). NP-specific Ab titers in TG or WT mice were measured as described (3). NP2-BSA or NP1-BSA (1 μg/ml; Biosearch Technologies) in PBS was used to coat Multiscreen plates (Millipore), and the plates were later blocked with 2% skim milk in PBS.

**Flow cytometry**

The procedure for flow cytometric analysis was according to a previous report (19). Abs used in flow cytometric analysis were purchased from BD Biosciences, unless otherwise mentioned, and they are: allophycocyanin– or peridinin chlorophyll–conjugated anti-human CD38 (clone HB7), PE-conjugated anti-human IgD (clone IA6-2), PerCP-Cy5.5-conjugated anti-B220 (clone 3D4), FITC-conjugated anti-mouse GL7 (clone GL7), allophycocyanin-conjugated anti-mouse IgG1 (clone X56), allophycocyanin–Cy7–conjugated β2-microglobulin and Rp32 mRNAs were used for internal normalization for human and mouse samples, respectively. The β2-microglobulin, PRDM1–, ABF-1–, PAX-5–, BCL6–, XBP-1–, Msc–, and Rp32-specific TaqMan primer and probe sets were purchased from Applied Biosystems. The primer sequences used in SYBR Green method are listed in Supplemental Table I.
anti-mouse B220 (clone RA3-6B2), PE-Cy7– or FITC-conjugated anti-mouse CD38 (clone 90, BioLegend), FITC-conjugated anti-mouse CD79b (clone 55B5; BioLegend), and CD20 (clone M11; BioLegend) were used to define memory B cells. For quantification of GC B cells, spleen sections from three WT or ITG mice were analyzed. In each section, three GCs were counted. For histological analysis, spleen sections were dewaxed and incubated with retrieval solution (pH 9; DakoCytomation). After blocking with 3% human serum (Invitrogen), slides were incubated with biotinylated peanut agglutinin (PNA; 1:100; Vector Laboratories) at 4°C overnight and then immersed in VECTASTAIN ABC (Vector Laboratories) followed by color development with diaminobenzidine staining. After washing with tap water, slides were incubated with anti-B220 (BD Pharmingen) at 4°C overnight. Immunoreactive signals were visualized by incubation of anti-rat IgG conjugated with alkaline phosphatase (Vector Laboratories) followed by color development with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche). For quantification of GC B cells, spleen sections from three WT or ITG mice were analyzed. In each section, three GCs were randomly selected for counting PNA+ cells in fixed area (80 × 50 μm).

**Microarray**

Three to five micrograms total RNA was used per cDNA microarray. The following procedures and data analysis were performed essentially as previously described (25). The microarray dataset from this study is deposited at the National Center for Biotechnology Information Gene Expression Omnibus with accession number GSE26583 (http://www.ncbi.nlm.nih.gov/geo/).

**Statistics**

Statistical significance was analyzed by the paired t test for human samples or unpaired t test for mouse study. Data shown are the mean ± SD or the mean ± SEM as indicated. Significance of p values was as follows: *p < 0.05, **p < 0.005, ***p < 0.001.

**Results**

**ABF-1 is predominantly expressed by memory B cells and altered expression of ABF-1 in memory B cells affects human plasma cell formation in vitro**

Human blood B cells treated with IL-21 and anti-CD40, which mimics the help from Th cells, undergo extensive proliferation, class switching, and Ab secretion (26, 27). Using this differentiation model, we examined the expression of ABF-1. As reported (27), PRDM1 mRNA levels were induced (Fig. 1A). We found that following stimulation with IL-21 and anti-CD40, ABF-1 mRNA levels declined by day 6 (Fig. 1B). Given that human peripheral B cells consist of naive B cells and memory B cells, and they can be separated on the basis of the absence or presence of surface CD27 expression, respectively (28), we wondered if ABF-1 is expressed in particular subsets of B cells. PRDM1 mRNA was induced in both naive and memory B cell–derived cultures treated with IL-21 and anti-CD40 (Fig. 1C). Notably, the basal levels of ABF-1 mRNA in freshly isolated memory B cells were significantly higher than the levels in naive B cells (Fig. 1C); the levels of ABF-1 mRNA in memory B cell–derived cultures increased on the first day and then declined thereafter (Fig. 1C).

We then examined if ABF-1 is involved in the formation of Ab-secreting cells that were differentiated from human peripheral blood memory B cells. We found that reduced generation of IgGlo CD38↑ cells and decreased production of IgG were detected in stimulated memory B cell–derived cultures expressing ABF-1–GFP compared with those expressing GFP alone (Fig. 1D–F). Reversely, we assessed if knockdown of ABF-1 expression in human blood memory B cells with a lentiviral vector expressing short hairpin RNA (shRNA) against ABF-1 (Fig. 1G), affects the formation of Ab-secreting cells. Our results show that knockdown of ABF-1 led to increased frequency of CD38↑ cells (Fig. 1H) and elevated numbers of IgG-secreting cells (Fig. 1I) 3 d after stimulation. The effect of ABF-1 on the production of IgG-secreting cells is linked with the alternation of cell proliferation, because the frequencies of BrdU+ were decreased in ABF-1–GFP-overexpressing cells (Fig. 1J, left panel) and increased in ABF-1 knockdown cells (Fig. 1K, left panel). However, modulation of the levels of ABF-1 did not affect cell apoptosis (Fig. 1J, 1K, right panel). Together, these results suggest that ABF-1 may limit the proliferation of stimulated memory B cells and the differentiation of plasma cells.

**Identification of genes regulated by ABF-1**

We next sought to examine the mechanisms by which ABF-1 possesses the activity to prohibit plasma cell differentiation. It is noted that the inhibitory effect of ABF-1 on the formation of Ab-secreting cells is associated with the higher level of BCL6, PAX5, and reduced XBP-1 mRNA, but the mRNA level of PRDM1 in ABF-1–GFP-expressing cells is not affected (Fig. 2A), suggesting that, independently of regulating PRDM1, ectopically expressed ABF-1 may promote the expression of mature B cell or GC B genes that prohibit plasma cell differentiation.

Furthermore, the differential gene expression profiles between a human B lymphoblastoid SKW6.4 cell line (SKW) that expressed ABF-1–GFP versus those that expressed GFP alone were examined by microarray analysis. Pairwise comparison of genes differentially expressed between GFP+ and ABF-1–GFP+ SKW cells revealed a limited number of genes for which expression was changed by >2-fold in two of our three independent microarray experiments (Fig. 2B). Thirty-seven genes were upregulated, and 10 genes were downregulated by ABF-1 (Supplemental Table I). The expression of several genes, including FLI1, FCRL2, FCRL4, FCRL5, SELL, and PAX5, was validated by RT-QPCR in SKW cells (Fig. 2C). However, in primary human memory B cell–derived culture, we only observed that FLI1 is downregulated and FCRL4 is upregulated in sorted ABF-1–GFP+ cells as compared with sorted GFP+ cells (Fig. 2D). FLI1 was of particular interest because it is known to play a role in normal cell proliferation and malignant transformation (29). Thus, downregulation of FLI1 by ABF-1 may, at least in part, explain why ectopic expression of ABF-1 limits the proliferation of stimulated memory B cells. Additionally, increased expression of FCRL4 by ABF-1 is also consistent with the notion that FCRL4 is predominantly expressed by memory B cells (30).

**ABF-1 is a direct target of Blimp-1**

On day 4, at least three cellular subsets were generated in memory B cell–derived cultures, and they can be grouped according to the surface level of CD20 and CD38 (Fig. 3A). According to a previous report (31), CD20+CD38↑ cells are the activated B cells that show 9 ± 3% in the S phase in our cultures, whereas CD20+CD38↓ cells showing 19 ± 5% in the S phase are the cycling plasmacytic cells termed plasmablasts. PRDM1 mRNA is expressed at the highest level in sorted CD20+CD38↑ plasmablasts from day 4 cultures as compared with those in sorted CD20+CD38↓ activated B cells from day 4 and memory B cells from...
ABF-1 is expressed in memory B cells and negatively regulates the production of Ab-secreting cells during human plasma cell differentiation. RT-QPCR analysis of *PRDM1* (A) and *ABF-1* (B) mRNA in IL-21- and anti-CD40-stimulated human peripheral B cells on the indicated days. (C) RT-QPCR analysis of *PRDM1* and *ABF-1* mRNA in IL-21- and anti-CD40-stimulated human peripheral naïve B cell-derived (top panel) or memory B cell-derived (bottom panel) cultures on the indicated days. The fold of expression was compared with the level of indicated genes in naïve B cells on day 0. (D) Immunoblotting by using anti-ABF-1 for ABF-1–GFP expressed from the lentiviral vector in SKW cells. Tubulin was used as a protein loading control. (E) Forced expression of ABF-1 reduces the formation of IgD<sup>−</sup>CD38<sup>hi</sup> cells produced by stimulated memory B cell–derived cultures. On days 6 to 7, GFP<sup>+</sup> and ABF-1–GFP<sup>+</sup> gated cells were subjected to the analysis of surface IgD and CD38 expression by flow cytometry. (F) GFP<sup>+</sup> and ABF-1–GFP<sup>+</sup> cells sorted from (E) on day 5 were cultured for 2 more d before collecting supernatants for ELISA of IgM (left panel) and harvesting cells for ELISPOT of IgG (right panel) levels. (G) RT-QPCR analysis of the levels of *ABF-1* mRNA in day 3 stimulated human memory B cells transduced with two lentiviral vectors producing different shRNA against ABF-1 (shABF-1) or control shRNA containing scramble sequences (shCtrl). (H) Frequency of CD38<sup>hi</sup> cells in the GFP<sup>+</sup> gate on day 3 after stimulation as determined by flow cytometric analysis. (I) ELISPOT determining IgG-producing cells from shABF-1– or shCtrl-transduced memory B cells stimulated with IL-21 and anti-CD40 for 3 d. (J) ABF-1 affects memory B cell proliferation, but not apoptosis, after stimulation with IL-21 and anti-CD40 for indicated days. Frequencies of BrdU<sup>+</sup> cells (left panel) and Annexin V<sup>+</sup> cells (right panel) in GFP<sup>+</sup> or ABF-1–GFP<sup>+</sup> gate as determined by flow cytometric analysis. (K) Knockdown of ABF-1 affects memory B cell proliferation, but not apoptosis, on day 3 after stimulation. Frequencies of BrdU<sup>+</sup> cells (left panel) and Annexin V<sup>+</sup> cells (right panel) in GFP<sup>+</sup> gate. Data in (A)–(C) and (G)–(K) represent the mean ± SD (n = 3, except n = 4 in H). *p < 0.05, **p < 0.005, ***p < 0.001.

day 0 cultures (Fig. 3B, left panel). It is noted that the expression of *ABF-1* mRNA is reduced in cycling plasmablasts, which is inversely correlated with the level of *PRDM1* mRNA (Fig. 3B, right panel). The inverse expression profiles of *ABF-1* and *PRDM1* in differentiating human B cell subsets prompted us to examine if *ABF-1* is directly repressed by Blimp-1. Indeed, ectopic expression of Blimp-1 by a lentiviral vector that expresses the GFP–Blimp-1 fusion protein in SKW cells reduced *ABF-1* mRNA and protein levels (Fig. 3C, 3D). We next compared the genomic sequences 3 kb upstream of the transcription start site of *ABF-1* with the known Blimp-1 consensus binding sequences (32) and found five putative Blimp-1 binding sites in the 5′ flanking region of *ABF-1* (Fig. 3E). A previously established stably transfected WI-L2 cell line that expresses a FLAG-tagged Blimp-1–ERD fusion protein under the control of the heavy metal–inducible metallothionein promoter was used to determine if Blimp-1 binds to *ABF-1* after the addition of CdCl<sub>2</sub> and 4-hydroxytamoxifen (4-OHT) (10). As shown with ChIP, Blimp-1 bound to site 4 and site 5 in the 5′ flanking region of *ABF-1* (Fig. 3F). A control WI-L2–transfected cell line expressing ERD alone and a locus in the 24-kb region of *ABF-1* served as negative controls. Furthermore, EMSAs demonstrated that bands representing sites 4 and 5 were shifted by Blimp-1 in nuclear extract isolated from 293T cells overexpressing Blimp-1 (Fig. 3G). The shifted complex was abolished by addition...
of indicated molar excess of unlabeled oligonucleotides containing sites 4 and 5, but not by site 4 or site 5 mutant oligonucleotides. Furthermore, the shifted complex was supershifted by a Blimp-1–specific mAb, whereas a mouse IgG isotype control Ab did not supershift the complex (Fig. 3G). It is noted that treatment with IL-21 plus anti-IgM plus anti-CD40 showed a synergistic effect on the induction of Msc mRNA (Fig. 4B). Likewise, Msc protein was induced in mouse splenic B cells 1 and 3 d after stimulation (Fig. 4C). On day 5, Msc protein was reduced, which is linked with the robust induction of Blimp-1 protein (Fig. 4C). These data suggest that Msc is induced by IL-21 and CD40L signals provided by Th cells in GC B cells and expressed by memory B cells in mouse.

**Induction of ABF-1 during primary immunization enhances the formation of Ag-specific memory B cells**

To determine the function of ABF-1 in B cell immune responses in vivo, we generated TG mice that expressed ABF-1 in B cells in an inducible manner to avoid the potential effect of ABF-1 on the function of E2A during early B cell development. cDNA of ABF-1 was fused to estrogen receptor (ER) cDNA, and its transcription function of the ABF-1–ER fusion protein in B cells could be induced via i.p. injection with 4-OHT. We obtained two mouse lines (lines 6 and 24) that expressed ABF-1–ER in splenic B cells (Fig. 5A, 5B), but the experiments were conducted in the line that expressed higher levels of ABF-1–ER, line 24, unless otherwise specified. B cell development and Ab responses following immunization were comparable in littermate control WT and TG mice (Supplemental Fig. 1A–C). After receiving several i.p. injections with IL-21 plus anti-IgM plus anti-CD40 showed a synergistic effect on the induction of Msc mRNA (Fig. 4B). Accordingly, ITG mice also developed a greater number of GCs because more PNA+ B cells sorted from immunized mice had low levels of Msc mRNA (Fig. 4A). Furthermore, Msc mRNA was induced in mouse splenic B cells stimulated in vitro with IL-21 and anti-CD40 but not with LPS or anti-IgM alone (Fig. 4B). It is noted that treatment with IL-21 plus anti-IgM plus anti-CD40 showed a synergistic effect on the induction of Msc mRNA (Fig. 4B). Moreover, Msc protein was induced in mouse splenic B cells 1 and 3 d after stimulation (Fig. 4C). On day 5, Msc protein was reduced, which is linked with the robust induction of Blimp-1 protein (Fig. 4C). These data suggest that Msc is induced by IL-21 and CD40L signals provided by Th cells in GC B cells and expressed by memory B cells in mouse.

**Induction of ABF-1 during primary immunization enhances the formation of Ag-specific memory B cells**

We next assessed the generation of GC B cells, Ag-specific memory B cells, and plasma cells after challenge with NP-KLH in ITG mice. Fig. 5E shows that ITG mice elicited more B220+ GL7+CD38– GC B cells than did WT mice. Accordingly, ITG mice also developed a greater number of GCs because more PNA+ B220+ GC B cells were detected in the spleen of ITG mice 2 wk after immunization compared with those in the spleen of WT mice (Fig. 5F). Furthermore, elevated frequency and numbers of
NP-specific memory B cells (B220+NP+IgG1+CD38+) were represented in the spleen of ITG mice 2 wk after immunization when compared with those of WT mice (Fig. 5G). These data suggested that induction of ABF-1 during thymus-dependent immune response may promote the formation of Ag-specific GC B and memory B cells. Similar results were found in another ITG line (line 6) that expressed lower levels of inducible ABF-1–ER (Supplemental Fig. 1D).

Interestingly, although there were more GC B cells in ITG mice, the frequency of BrdU+ proliferating cells in the B220+CD38+GL7+ GC B cell gate was lower in the spleen of ITG mice than of WT mice, even though the frequency of BrdU+ in non–B cells (B220−) isolated from splenocytes of WT and ITG mice was comparable (Fig. 5H). These contradictory data showing increased numbers but reduced proliferation of GC B cells in ITG mice suggest that ABF-1 may have additional functions that ultimately maintain GC B cells.

Together, by using an inducible ABF-1 TG model, we demonstrated that induced ABF-1 promotes the formation of GC and memory B cells after immunization with NP-KLH.

Induction of ABF-1 during primary and secondary immunization prevents plasma cell formation in mouse

With regard to the generation of plasma cells, we found that the number of plasma cells that secreted either NP 52-specific IgG or NP4-specific IgG in the spleen of NP-KLH–immunized ITG mice was compromised as compared with those of WT mice 14 d after immunization (Fig. 6A). Congruently, the total NP52-specific IgG titer in sera from ITG mice was less than that in WT mice 14 and 21 d after immunization (Fig. 6B). Additionally, compared with WT mice, the serum levels of high-affinity NP4-specific IgG in ITG mice were reduced 7 and 14 d after immunization (Fig. 6C), but NP-specific IgM levels in sera did not change significantly in...
ITG mice (Fig. 6D). However, the ratio of NP4/52 was comparable between WT and ITG mice (Fig. 6E).

Given that ectopic expression of ABF-1 inhibited the generation of human plasma cells derived from memory B cells in vitro, we assessed whether induction of ABF-1 activity in TG mice during secondary immunization (2ITG) could recapture the decreased generation of plasma cells (Fig. 7A). Although the frequency of GC B cells was comparable in WT and TG mice before Ag recall, the frequency of GC B cells was increased in 2ITG mice (Fig. 7B), which is similar to the effect of ABF-1 on enhancing GC B cell formation in primary immunization. The numbers of total NP4-specific IgG-producing cells and NP2-specific IgG-secreting cells were significantly reduced in the spleen of 2ITG mice 7 d after Ag recall (Fig. 7C). Furthermore, the frequency of emerging plasma cell precursors or preplasma memory B cells, defined as NP+CD79b+B220-CD138+ (3, 33), in the spleen of 2ITG mice 5 d after Ag recall was consistently reduced (Fig. 7D, 7E) as compared with those in WT mice, even though the fre-

![Figure 4](http://www.jimmunol.org/Downloaded.png)

**FIGURE 4.** *Msc* is induced by Tfh-mediated signals. (A) RT-QPCR analysis of *Msc* mRNA levels in the indicated sources of cells/tissue. Ag-specific splenic GC B (B220+NpIgG1+CD38+GL7+), memory B (B220+NpIgG1+CD38+GL7+), and plasma (B220loCD138+) cells were sorted by flow cytometry 14 d after NP-KLH immunization. The fold expression was compared with the level of *Msc* in naive splenic B220+ B cells. Muscle sample is the positive control. (B) RT-QPCR shows the mRNA level of *Msc* in mouse splenic B cells treated with the indicated stimuli at the indicated time points. (C) Immunoblot shows the expression of Blimp-1 and Msc in mouse splenic B cells treated with IL-21, anti-CD40, and anti-IgM on the indicated days. Tubulin was used as a loading control. Data are from a single experiment that is representative of at least three experiments. Data in (A) and (B) represent the mean ± SEM (n = 3).

![Figure 5](http://www.jimmunol.org/Downloaded.png)

**FIGURE 5.** Induction of ABF-1 in vivo alters GC B and memory B cell formation. (A) Immunoblot analysis using an Ab against ABF-1 shows the expression of ABF-1-ER in the splenic B cells of TG mouse lines 6 and 24 but not in line 28 or WT control. Tubulin was used as an internal loading control. (B) Immunoblot analysis shows the expression of ABF-1–ER in B cells, but not in T cells or other tissues, from line 24. (C) Experimental approach. TG and WT mice were i.p. injected with 4-OHT eight times at 2-d intervals starting at 1 d before immunization with NP-KLH. (D) Immunoblotting shows the translocation of ABF-1–ER from the cytoplasm (Cyto) to the nucleus (N) of splenic B cells of ITG mice. TG mice were i.p. injected with oil control or 4-OHT as described in (C). On day 14, splenic B cells were subjected to fractionation of nuclear and cytosolic compartments for immunoblotting with anti–ABF-1. Tubulin and histone deacetylase 2 (HDAC) blots were used as the controls for cytoplasmic and nuclear fraction isolation, respectively. (E) Flow cytometry of GC B cells with the indicated Abs using splenic B cells from WT littermate control and ITG mice 14 d after immunization with NP-KLH. The frequency of GC B cells in B220+ gate is shown. (F) Immunohistochemistry staining with anti-B220 and PNA lectin on spleen sections of WT and ITG mice 14 d after immunization with NP-KLH. Scale bars, 300 μm. Quantification result is shown in the right panel. (G) A representative flow cytometric analysis of the frequency of B220+NpIgG1+CD38+IgG1+ memory B cells from mice as described in (E) is shown. Data represent the mean ± SEM (n = 5). (H) Fewer BrdU+ GC B cells, but not non-B cells, in ITG mice. Flow cytometric analysis of the frequency of BrdU+ splenocytes within B220+GL7+CD38– GC B cell and B220– non-B cell gates. Horizontal bars in (E) and (H) indicate the mean (n = 5 and 7, respectively). *p < 0.05, **p < 0.005, ***p < 0.001.
quency of memory B and plasma cell precursors was comparable in WT and TG mice before secondary immunization (Fig. 7E). These data supported our results from human plasma cell differentiation in culture showing that overexpression of ABF-1 in stimulated memory B cells reduced the formation of plasma cells.

Lastly, we wish to examine if ITG mice indeed developed more memory B cells than WT mice did after immunization. We adoptively transferred plasma cell–depleted splenocytes isolated from either WT or ITG immunized mice (CD45.2) to recipient mice (CD45.1), followed by boosting of recipient mice with NP–KLH (Fig. 7F). Results in Fig. 7G showed that mice received plasma cell–depleted splenocytes from ITG mice produced more NP52–specific IgG than mice received cells from WT mice. Consistently, at day 6 after adoptive transfer, mice (CD45.1) received plasma cell–depleted splenocytes from ITG mice had more B220−CD138+CD45.2+ plasma cells in bone marrow than mice received WT cells (Fig. 7G), supporting the notion that ITG mice generated more memory B cells after immunization.

Discussion

In this study, we provide evidence showing that ABF-1 is directly suppressed by Blimp-1 based on several of findings: 1) ectopic expression of Blimp-1 reduced endogenous ABF-1 mRNA and protein levels; 2) Blimp-1 bound to two sites in the 5′ flanking region of ABF-1 as shown by the ChIP assay, EMSA, and DNA pulldown assay; and 3) ABF-1 promoter activity was repressed by Blimp-1 in a site- and dose-dependent manner in cotransfected cells, as shown by the luciferase reporter assay. A minimal ABF-1 promoter was previously reported to be −182 bp upstream of the transcription start site (34). We found two elements located in the 5′ flanking region of ABF-1 that were responsive to Blimp-1 expression. One was located at −2850 to −2841 (site 5), and the other was at −2246 to −2235 (site 4). Both are further upstream of the previously reported ABF-1 minimal promoter. In fact, two other sites that are near −2118 to −2073 (site 2 and site 3) also contained potential Blimp-1 binding sequences. Using the ChIP assay would not be practical for determining the precise element(s) in the cluster of sites 2–4 that were directly bound by Blimp-1. We reasoned that site 4 in this cluster was the predominant Blimp-1 binding site because the activity of a luciferase reporter fused with the ABF-1 promoter carrying mutations at both sites 2 and 3 was still suppressed by Blimp-1 (Supplemental Fig. 2A). However, as ABF-1 regulation and its B cell–restricted control elements have not been fully characterized, it is possible that Blimp-1 regulates ABF-1 through additional sites.

A role for ABF-1 in blocking the B cell phenotype in Hodgkin lymphoma has been reported (16). In this study, we showed a previously unknown function for ABF-1 in normal B cells. ABF-1 was shown to be induced following treatment of peripheral human B cells with Staphylococcus aureus Cowan 1 (SAC) (14). In this study, in human memory B cell culture, ABF-1 mRNA levels were increased in the initial phase of memory B cells in culture when stimulated with IL-21 and anti-CD40, which mimics Th cell contact (1). In mouse splenic B cell culture, Msc mRNA and protein were as well induced by IL-21 and anti-CD40. The induction of Msc mRNA was synergistically induced by the combined treatment with IL-21, anti-CD40 and anti-IgM despite that anti-IgM treatment alone decreased Msc mRNA. Additionally, inhibition of NF-κB, but not p38 MAPK and ERK, signaling blocks the induction of Msc mRNA by the treatment with IL-21, anti-CD40, and anti-IgM (Supplemental Fig. 2B). The reasons why SAC, a mitogen capable of triggering human B cell proliferation and activation through cross-linking of surface Ig (35), induced ABF-1 in human B cells, but anti-IgM reduced Msc in mouse B cells are still unknown. It is possible that during the GC reaction in vivo, some B cells that acquired Th-mediated signals for higher ABF-1/Msc expression commit to becoming memory B cells.

In this study, we provide evidence showing that ABF-1 prevented the formation of IgG-secreting plasma cells by both the primary human memory B cell culture and the TG mouse model. We
reasoned that reduced IgG production may result from a negative role for ABF-1 in the differentiation of memory B cells into IgG-secreting plasma cells. High-affinity GC B cells are selected to form plasma cells (37). However, the NP4/NP52 ratio was comparable between WT and ITG mice after immunization with NP-KLH. Thus, we suspect that ABF-1 is involved in the machinery that controls the maintenance of GC B toward memory B cells, thereby prohibiting the differentiation of Ab-secreting cells. IgM-bearing memory B cells can be derived from the early stage of GC reaction (38) or independently of GCs (39). Although ABF-1 blocked the formation of human IgM-secreting cells in some culture, ITG mice produced normal amounts of Ag-specific IgM in primary immunization. Using our approach, we could not exclude the possibility that ABF-1 activity was not effectively induced by 4-OHT injections prior to the formation of IgM-secreting cells or IgM-bearing memory B cells, as both types of cells were generated early during primary immune responses. Although the expression levels of ABF-1 are low in naive B cells in both human and mouse, it is also plausible that ABF-1 negatively regulates naive B cell differentiation into IgM-secreting plasma cells. Therefore, the role of ABF-1 in the production of IgM awaits to be clarified.

What are the molecular mechanisms underlying ABF-1 suppression of plasma cell differentiation? ABF-1 appears to increase PAX5 and BCL6 transcription, likely through an indirect mechanism because ABF-1 is a transcriptional repressor (14). The ABF-1–mediated reduction of XBP-1 may, at least in part, result from increased PAX5 expression, because XBP-1, the gene required for Ab secretion in plasma cell differentiation (8), is suppressed by PAX5 (40). PAX5 is essential for B cell lineage commitment and maintenance of B cell identity (41). Downregulation of PAX5 during plasma differentiation is necessary and sufficient for plasma cell differentiation (9, 42, 43). As ABF-1 upregulates PAX5, it is conceivable that ABF-1 may be suppressed to allow plasma cell formation. Furthermore, ABF-1 elevated the expression of BCL6, the important regulator for GC formation (44), which is reconciled by our results from NP-KLH–immunized ITG mice showing that ABF-1 promotes the generation of GC B cells. Interestingly, our microarray data revealed that several FCRLs were upregulated by ABF-1. Among them, FCRL2 and FCRL4 are predominantly expressed by memory B cells (30), supporting again the notion that ABF-1 expression is relevant to memory B cell fate. Although FCRL4 has been shown to negatively regulate BCR-mediated activation (45), its role in memory B cells awaited future studies. Another target obtained from our microarray data is FLI1, which has been implicated in accelerating cell proliferation (29). Its overexpression in mice leads to B cell hyperplasia and a spontaneous systemic lupus erythematosus–like disease (46, 47). More importantly, the proliferation of B cells from Flil-deficient mice is impaired (48). These combined findings suggested that the diminished cell proliferation resulting from ec-
topic expression of ABF-1 in human memory B cells may be partly due to reduced FLI1 expression. Furthermore, ABF-1–mediated suppression of FLI1 may at least partly explain why memory B cells manifest cell-cycle arrest (49), although it requires further experiments to test this hypothesis.

In summary, in this study, we propose a gene regulatory pathway for Blimp-1 and ABF-1 during memory B cells versus plasma cell fate commitment. Th-primed GC B cells that acquire the expression of ABF-1 may differentiate toward the memory B cell fate, whereas Blimp-1–expressing cells may bias the formation of plasma cells that in turn show the reduced expression of ABF-1. During the differentiation of plasma cells from memory B cells, the induced Blimp-1 suppresses ABF-1, leading to derepression of ABF-1 downstream genes, such as FLI1, that is involved in cell proliferation. As a consequence, it may permit further rounds of proliferation for allowing the production of Ab-secreting cells. Further understanding of the role of ABF-1 in memory B cells combined with modulating the Blimp-1/ABF-1 regulatory pathway may aid in boosting immune responses following vaccination.

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Disclosures

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References


