Batf coordinates multiple aspects of B and T cell function required for normal antibody responses

Briana C. Betz,1 Kimberly L. Jordan-Williams,1 Chuanwu Wang,2 Seung Goo Kang,2 Juan Liao,1 Michael R. Logan,2 Chang H. Kim,2,3 and Elizabeth J. Taparowsky1,3

1Department of Biological Sciences, 2Department of Comparative Pathobiology and 3Purdue University Center for Cancer Research, Purdue University West Lafayette, IN 47907

Batf belongs to the activator protein 1 superfamily of basic leucine zipper transcription factors that includes Fos, Jun, and Atf proteins. Batf is expressed in mouse T and B lymphocytes, although the importance of Batf to the function of these lineages has not been fully investigated. We generated mice (Batf<sup>−/−</sup>) in which Batf protein is not produced. Batf<sup>−/−</sup> mice contain normal numbers of B cells but show reduced numbers of peripheral CD4<sup>+</sup> T cells. Analysis of CD4<sup>+</sup> T helper (Th) cell subsets in Batf<sup>−/−</sup> mice demonstrated that Batf is required for the development of functional Th type 17 (Th17), Th2, and follicular Th (Tfh) cells. In response to antigen immunization, germinal centers were absent in Batf<sup>−/−</sup> mice and the maturation of Ig-secreting B cells was impaired. Although adoptive transfer experiments confirmed that this B cell phenotype can be driven by defects in the Batf<sup>−/−</sup> CD4<sup>+</sup> T cell compartment, stimulation of Batf<sup>−/−</sup> B cells in vitro, or by a T cell–independent antigen in vivo, resulted in proliferation but not class-switch recombination. We conclude that loss of Batf disrupts multiple components of the lymphocyte communication network that are required for a robust immune response.

The development of the various lymphoid lineages is regulated by many transcription factors, including the dimerizing basic leucine zipper (bZIP) proteins collectively known as activator protein 1 (AP-1; Wagner and Eferl, 2005). The classical AP-1 transcription factor consists of a Jun:Fos heterodimer, although tissue-restricted bZIP proteins, including several of the Maf, Atf, and Batf proteins, provide alternative partner choices for Fos and/or Jun (Eferl and Wagner, 2003). Properties conferred on AP-1 by dimer composition and posttranslational modifications influence the DNA targets bound by AP-1 and, in some cases, convert what is normally a transcriptional activator into a transcriptional repressor (Eferl and Wagner, 2003; Hess et al., 2004; Amoutzias et al., 2006). It is not surprising, therefore, that AP-1 plays roles in cell growth, differentiation, and apoptosis (Hess et al., 2004) and that deregulated AP-1 activity is a feature of many pathologies, including cancer and neurological diseases (Eferl and Wagner, 2003; Raivich and Behrens, 2006).

© 2010 Betz et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-nd/3.0/).
together, these data indicated that Batf was required for the development of thymic T cells and for the differentiation of peripheral B cells into antibody-producing cells.

**RESULTS AND DISCUSSION**

**Decreased numbers of peripheral CD4+ T cells in Batf<sup>Z/ΔZ</sup> mice**

To examine the role of Batf in lymphocyte development, we first generated Batf knockin (Batf<sup>KI</sup>) mice in which exon 3, the ZIP coding region of Batf, is expressed with a C-terminal hemagglutinin antigen (HA) epitope tag (Fig. 1 A). This modified exon and the Pyk-neo cassette used for ES cell selection are flanked by loxP sites, permitting the excision of both elements using Cre recombinase. Batf<sup>KI</sup> mice were crossed to Cre-expressing mice (Elisa-Cre), producing heterozygous (Batf<sup>Z/ΔZ</sup>) mice which were crossed to generate homozygous Batf<sup>Z/ΔZ</sup> mice and littermate Batf<sup>+/+</sup> and Batf<sup>/−/+</sup> mice for comparison (Fig. 1 A and B). Batf<sup>Z/ΔZ</sup> mice do not produce a functional Batf bZIP protein. Immunoblots using Batf<sup>Z/ΔZ</sup> splenocyte extracts and anti-HA antiseraum failed to detect a protein (Fig. 1 C). As predicted, semi-quantitative PCR (qPCR) analysis of RNA isolated from Batf<sup>Z/ΔZ</sup> splenocytes using several primer sets detected transcripts representing exons 1 and 2 but no transcript specifying the Batf ZIP domain (Fig. S1 A and B).

**Batf mRNA and protein are expressed in mouse B cells**

and in all major T cell subsets examined, with the exception of double-positive thymocytes (Williams et al., 2001) which, interestingly, lack all AP-1 activity (Rincón and Flavell, 1996). Mice expressing human BATF throughout T cell development in the thymus (p56<sup>Δ/Δ</sup>HA-BATF) possess normal numbers of CD4+ and CD8<sup>+</sup> T cells but are impaired in the development of V<sub>α</sub>11 NKT cells (Williams et al., 2003; Zullo et al., 2007). To determine if Batf or T cell development is altered by the absence of Batf, cells from the thymus, spleen, and Peyer’s patches (PPs) of Batf<sup>/−/−</sup> and Batf<sup>Z/ΔZ</sup> mice were analyzed by flow cytometry. No significant difference in thymic T cell populations was observed (Fig. S2 A). In the periphery, a trend toward a decreased number of T cells and an increase in B cell numbers was noted, yet statistical significance was established only for CD4<sup>+</sup> T cells (Fig. 1 D). In agreement with a recent study (Schraml et al., 2009), we did not detect increases in any T cell subset in Batf<sup>Z/ΔZ</sup> mice, including V<sub>α</sub>11 NKT cells (unpublished data). This was unexpected based on the NKT cell–deficient phenotype of p56<sup>Δ/Δ</sup>HA-BATF mice (Williams et al., 2001; Zullo et al., 2007) and on experimental evidence that BATF inhibits cell proliferation in different contexts (Echlin et al., 2000; Williams et al., 2001; Senga et al., 2002; Thornton et al., 2006). Instead, this supports a model where the overexpression of an AP-1 inhibitor, such as Batf, can have a dramatic impact on cells, whereas the impact of deleting Batf might be masked by the compensatory actions of other AP-1 inhibitors (e.g., Batf3, JunD, FoxB, and Atf3; Hess et al., 2004). Although a comparative profile of all AP-1 proteins expressed by various lymphocyte lineages has yet to be compiled, Batf and Batf3 are coexpressed in mouse Th1 cells, for example (Williams et al., 2003; Hildner et al., 2008). In this regard, transgenic mice in which either of these proteins is overexpressed during T cell development share phenotypes, including the NKT cell defect (unpublished data), whereas the absence of Batf or Batf3 has an impact on other cell types (Hildner et al., 2008; Schraml et al., 2009). Thus, it is the unique functions of Batf that will be revealed by a thorough analysis of Batf<sup>Z/ΔZ</sup> mice.

**Th2 and Th17 cells require Batf**

The decrease in peripheral CD4<sup>+</sup> T cells associated with Batf deficiency prompted us to further investigate this phenotype. CD4<sup>+</sup> T cells represent multiple T helper (Th) cell lineages (Zhou et al., 2009). To measure Th cell subsets in Batf<sup>/−/−</sup> and Batf<sup>Z/ΔZ</sup> mice, CD4<sup>+</sup> T cells isolated directly from spleen and PP were analyzed by flow cytometry after a brief stimulation. IFN-γ and IL-4 are well characterized markers of the Th1 and Th2 lineages, respectively, and no statistically significant difference was noted for either cell type (Fig. 2 A). In contrast, a dramatic underrepresentation of CD4<sup>+</sup> T cells expressing IL-17 (Th17 cells) was apparent in Batf<sup>Z/ΔZ</sup> mice (Fig. 2 A). A small but significant reduction in Foxp3<sup>+</sup> CD4<sup>+</sup> regulatory T (T reg) cells also was noted in Batf<sup>Z/ΔZ</sup> mice (Fig. S2 B).

To investigate if Batf deficiency affects the expression of genes that are markers for activated CD4<sup>+</sup> Th cell subsets, RNA was prepared from CD4<sup>+</sup> Batf<sup>/−/−</sup> and Batf<sup>Z/ΔZ</sup> splenocytes after stimulation with anti-CD3<sup>e</sup> mAb for 48 h. qPCR was used to quantify transcripts unique to Th17 (IL-21, IL-23R, and IL-17), Th1 (T-bet), Th2 (Gata3 and IL-4), and T reg (Foxp3) cells. Results confirm the underrepresentation of Th17 cells, the normal levels of Th1 cells, and the modest reduction of T reg cells in Batf<sup>Z/ΔZ</sup> mice (Fig. 2 B). Interestingly, although no significant change in Th2 cells was detected by flow cytometry (Fig. 2 A), the low levels of
IL-4 and Gata3 mRNA noted in Fig. 2 B suggest a role for Batf in Th2 responses. As confirmation that a difference in mRNA by this assay reflects a change in protein, ELISA was performed on media harvested from stimulated Batf+/+ and BatfΔZ/ΔZ splenocytes. Results showed that BatfΔZ/ΔZ cells secrete normal levels of IFN-γ, reduced levels of IL-4, and extremely low levels of IL-17 (unpublished data).

To compare the ability of Batf+/+ and BatfΔZ/ΔZ CD4+ T cells to respond to cues that polarize cells to distinct Th cell lineages, naive CD4+ T splenocytes, cultured under well defined Th1, Th2, Th17, and T reg cell conditions, were analyzed by flow cytometry. This general approach was used previously (Schraml et al. 2009) to demonstrate a role for Batf in Th17 differentiation. In agreement with those studies, we found that Batf+/+ and BatfΔZ/ΔZ cells were equally competent for Th1 differentiation (Fig. S2 C) and that the decreased levels of T reg cells noted in vivo did not reflect an inability of naive BatfΔZ/ΔZ T cells to differentiate to T reg cells in vitro (Fig. S2 D). Our results also confirmed that BatfΔZ/ΔZ cells cannot be directed toward the Th17 lineage under conditions where >40% of control Batf+/+ cells express IL-17 (Fig. S2 E). In contrast, attempts to assess Th2 polarization by flow cytometry produced inconsistent results for both Batf+/+ and BatfΔZ/ΔZ cells over several experiments, prompting us to rely on qPCR analysis of Th2 transcripts as an indicator of differentiation. qPCR with RNA from in vitro–polarized Th17 cultures was performed in parallel. As shown in Fig. 2 (C and D), when compared with control cells, BatfΔZ/ΔZ cells did not induce significant levels of either Th2- or Th17-specific transcripts. Although Schraml et al. (2009) did not describe a defect in polarized Th2 differentiation for their Batf-deficient cells, our results would indicate that there is, at minimum, a partial defect in the Th2 cell subset that contributes to a decreased level of IL-4 in BatfΔZ/ΔZ mice.
Impaired Ig production in Batf^{−/−} mice

IL-21 is required for the differentiation of Th17 cells and, in turn, is produced by Th17 cells (and other cell types) to stimulate IL-21–producing CD4+ Tfh cells and the B cell Ab response (King, 2009). Mice lacking IL-4 and the IL-21 receptor exhibit severe defects in Ab production (Ozaki et al., 2002). To test if the combined IL-4− and IL-21− deficient phenotype of Batf^{−/−} mice results in reduced Ig production, circulating IgM, IgG1, IgG2c, IgA, and IgE were quantified by ELISA. As shown in Fig. 3 A, when compared with Batf^{+/+} animals, Batf^{−/−} mice displayed a modest reduction in circulating IgM. Strikingly, the levels of all other Ig classes examined were barely detectable in Batf^{−/−} mice.

To test if Ig production in Batf^{−/−} mice remains low in the presence of antigen challenge, Batf^{+/+} and Batf^{−/−} mice were injected with sheep RBC (sRBC) or mock injected with PBS. After 7 d, serum was isolated and circulating Ig quantified by ELISA. Again, although anti-sRBC IgM was induced in both Batf^{+/+} and Batf^{−/−} mice, induction of IgG by Batf^{−/−} mice was only 26% of the control (Fig. 3 B). Immunohistochemistry (IHC) confirmed the low levels of IgG1 and IgG2c in spleens of Batf^{−/−} mice.
Figure 3. Dysgammaglobulinemia in Batf(+/−) mice. (A) ELISA was performed to measure the indicated Ig in sera of Batf(+/+) and Batf(−/−) mice. Shown are mean results from four mice per group (n = 4) assayed in duplicate. Error bars indicate SE, *, P < 0.05. (B) Sera from Batf(+/+) and Batf(−/−) mice, immunized with sRBC or mock injected with PBS, were used in ELISA to detect sRBC-specific IgM or IgG. Mean results from one (n = 3) of two experiments are shown. Error bars indicate SE, *, P < 0.05. (C) Spleen sections from mice in B (n = 3 for each genotype) were incubated with primary anti-IgG1 and anti-IgG2c Abs. Complexes were detected using biotinylated secondary Abs and Vectastain ABC reagent. Shown are representative images, counterstained with H (no E) and photographed at 40×. f, follicle. Bars, 50 µm. (D) Spleen sections from mice in B (n = 3 for each genotype) were stained with H + E (left) and photographed at 4x (bars, 250 µm) or 20x (insets; bars, 50 µm). GCs (arrows) were detected on additional sections (right) using biotinylated PNA and Vectastain ABC reagent, counterstained with H (no E; 20x; bars, 50 µm) or using biotinylated PNA, anti–mouse B220, DAPI, and fluorescently labeled secondary mAbs (60x; bars, 50 µm).
mice (Fig. 3 C). The morphology of additional spleen sections from sRBC-challenged Batf\(^{+/+}\) and Batf\(^{\Delta Z/\Delta Z}\) mice was examined (Fig. 3 D). Although hematoxylin and eosin (H + E) and peanut agglutinin (PNA) staining demonstrated the presence of GCs in Batf\(^{+/+}\) mice, PNA\(^{+}\) GCs were conspicuously absent in Batf\(^{\Delta Z/\Delta Z}\) mice.

**Figure 4.** Dysfunctional Tfh cells in Batf\(^{\Delta Z/\Delta Z}\) mice. (A) Cells from spleen and PP of Batf\(^{\Delta Z/\Delta Z}\) and Batf\(^{+/+}\) mice were stained with anti-CD4, anti-CD44, anti-CD62L, and anti-CXCR5 mAb (or isotype control) and Tfh cells detected by flow cytometry. Representative plots (n = 9) showing CXCR5 expression after gating on CD44\(^{+}\)CD4\(^{+}\)CD62L\(^{+}\) or CD44\(^{+}\)CD4\(^{+}\)CD62L\(^{+}\) cells are presented. (B) Datasets from A (n = 9) are plotted as the mean percentage of CD62L\(^{+}\)CXCR5\(^{+}\) of total CD4\(^{+}\) (left) or number of CXCR5\(^{+}\) cells per organ or per total mouse PP (right) with SE, *P < 0.05. (C) 5 \times 10^5 cells from PP were allowed to migrate to rmCXCL13 for 3 h. Migrated cells were stained with anti-CD4 and anti-CD62L mAbs and analyzed by flow cytometry. Migration is the number of migrating cells (+ ligand) minus the number of migrating cells (no ligand) expressed as a percentage of CD4\(^{+}\)CD62L\(^{+}\) or CD4\(^{+}\)CD62L\(^{+}\) cells in the starting population. Mean results from three experiments are shown (n = 3). Error bars indicate SE, *P < 0.05.

**Reduced Tfh cell number and function in Batf\(^{\Delta Z/\Delta Z}\) mice**

The production of high-affinity class-switched Ab relies on GC interactions between B cells and Tfh cells (King, 2009). Tfh cells are characterized by the expression of CXCR5 (CXC chemokine receptor 5), which directs the homing of Tfh cells to B cell follicles in the spleen and lymph nodes (Kim et al., 2001). There is strong evidence to suggest that IL-21 is critical for Tfh development and that the IL-21 produced by Tfh cells in GCs is essential for the B cell response (Nurieva et al., 2008; Vogelzang et al., 2008; King, 2009). To quantify Tfh cells in Batf\(^{+/+}\) and Batf\(^{\Delta Z/\Delta Z}\) mice, CD4\(^{+}\) T cells were stained with mAb specific for CXCR5 and CD62L and analyzed by flow cytometry (Fig. 4 A). Results show that in Batf\(^{\Delta Z/\Delta Z}\) mice, memory-type CD62L\(^{−}\)CXCR5\(^{+}\) Tfh cells are reduced by 70% in the spleen (Fig. 4 B, top) and by 90% in PP (Fig. 4 B, bottom). To examine if Tfh cells in Batf\(^{\Delta Z/\Delta Z}\) mice are functional, purified CD4\(^{+}\) T cells from PP were challenged in vitro to migrate to CXCL13, the CXCR5 ligand. Migrating cells were counted and expressed as a percentage of CD62L\(^{−}\)CXCR5\(^{+}\) cells in the initial suspensions. As shown in Fig. 4 C, ~25% of Batf\(^{+/+}\) Tfh cells were capable of chemotaxis, whereas <10% of Batf\(^{\Delta Z/\Delta Z}\) Tfh cells displayed this behavior. These results are further support for an essential role for Batf in CXCR5\(^{+}\) Tfh cells.

Batf deletion has an impact on multiple CD4\(^{+}\) T cell lineages and, in doing so, generates an environment unfavorable to a robust Ab response. To demonstrate the T cell dependence of this phenotype, adoptive transfer was used to reconstitute T cell–deficient mice with CD4\(^{+}\) T cells purified from Batf\(^{+/+}\) or Batf\(^{\Delta Z/\Delta Z}\) mice. After transfer, the mice were challenged with sRBC and, 8 d later, Tfh cells were quantified and sera assayed for Ig. As predicted, when compared with mice reconstituted with Batf\(^{+/+}\) T cells, the spleens and lymph nodes of mice reconstituted with Batf\(^{\Delta Z/\Delta Z}\) T cells were not populated with CD62L\(^{−}\)CXCR5\(^{+}\) cells (Fig. 5, A and B) and sera from these animals contained less sRBC–induced IgM and IgG1 (Fig. 5 C).

**Batf\(^{\Delta Z/\Delta Z}\) B cells do not express Aicda mRNA and do not undergo CSR**

To this point, our data implicate defects associated with several CD4\(^{+}\) T cell subsets as the underlying cause of Ig deficiency in Batf\(^{\Delta Z/\Delta Z}\) mice. On the other hand, because Batf is expressed in mouse B cells (Williams et al., 2001) and functions as an inducible growth regulator in human B cells (Johansen et al., 2003), the loss of Batf could impact B cell function as well. To examine this possibility, resting B cells from spleens of Batf\(^{+/+}\) and Batf\(^{\Delta Z/\Delta Z}\) mice were cultured in control medium or in medium containing LPS, with or without added IL-4. Cells were analyzed for proliferation by BrdU staining after 40 h and for surface and secreted Ig after 4 d. Batf\(^{+/+}\) and Batf\(^{\Delta Z/\Delta Z}\) B cells proliferated similarly after exposure to LPS or LPS and IL-4 (Fig. 6 A). B cells of both genotypes also expressed surface and secreted IgM under all three growth conditions (Fig. 6 B). Strikingly, although control cells stimulated with LPS and IL-4 decreased...
IgM production and began producing IgG1 and IgE. Batf<sup>ΔZ/ΔZ</sup> cells continued to produce high levels of IgM, indicating that Batf is required for efficient CSR.

The inability of Batf<sup>ΔZ/ΔZ</sup> B cells to undergo CSR after stimulation was characterized further using qPCR to examine the expression of key genes known to participate in the expression, CSR, and somatic hypermutation (Fairfax et al., 2008; Park et al., 2009).

To confirm that this in vitro result reflects a B cell defect in vivo, Batf<sup>ΔZ/ΔZ</sup> animals were injected with TNP-LPS. After 4 d, T cell–independent responses were assayed by ELISA and IHC. Although Batf<sup>ΔZ/ΔZ</sup> mice showed no T cell–dependent antigen response by either assay.

The recent work of Schraml et al. (2009) clearly demonstrated a role for Batf in Th17 cell differentiation and cytokine gene regulation. Our studies have confirmed that role and described additional roles for Batf in Thh and Th2 cells that are required for the generation of a robust T cell–dependent antigen response in vivo. Moreover, our studies have revealed a role for Batf in the intrinsic responsiveness of B cells to T cell–independent stimulation in vitro and in vivo. Future studies, in which we exploit our conditional Batf<sup>ΔZ</sup> allele to disrupt Batf function in specific lymphocyte compartments or during key developmental transitions, will allow us to further dissect the molecular details of these intriguing Batf–dependent phenotypes.

**Figure 5.** Limited response of Batf<sup>ΔZ/ΔZ</sup> CD4<sup>+</sup> T cells to T cell–dependent antigen. (A) CD4<sup>+</sup> T cells from Batf<sup>+/+</sup> or Batf<sup>ΔZ/ΔZ</sup> mice were injected into T cell–deficient mice, which were immunized with 5 × 10<sup>8</sup> sRBC. After 8 d, Tfh cells in spleen and lymph nodes were quantified as in Fig. 4 A. Representative plots (n = 3) are shown. (B) Mean number of CXCR5<sup>+</sup> cells from A per organ or per mouse lymph node (LN) is shown (n = 3). Error bars indicate SE, *P < 0.05. (C) ELISA using sera isolated from mice in A to detect sRBC-specific IgM and IgG1. Mean and SE are shown (n = 3), *P < 0.05.

**MATERIALS AND METHODS**

**Generation of Batf<sup>ΔZ/KI</sup> and Batf<sup>ΔZ/ΔZ</sup> mice.** Batf primers with a 5′ loxP sequence and a 3′ HA epitope coding sequence (+ stop) were used to amplify a region of intron 2 plus exon 3 (– stop) of the Batf gene. This fragment was cloned into pBS KS and modified by insertion of the Batf 3′ UTR, at SpeI and of a loxP-flanked Pgk-neomycin selection cassette at XbaI. The Batf<sup>KI</sup> sequence was excised using EcoRI and cloned into pBS KS/ARMs containing 3.5 kbp of 5′ and 2.7 kbp of 3′ Batf genomic DNA. This plasmid, pBS KS Cko<sub>2</sub>, was linearized and introduced into 129/SV mouse embryonic stem cells, and the correct targeting of drug-resistant clones was determined by PCR with forward (5′-GGAAGGATCAGCTTGTGCT-3′) and reverse primers to detect endogenous (5′-GGAAGGATCAGCTTGTGCT-3′) or recombined (5′-CGAGCATAGTGAGACGTGCTAC-3′) Batf<sup>KI</sup> genes. The Transgenic Mouse Core Facility of the Purdue University Center for Cancer Research produced germine chimeras which were crossed to C57BL/6 mice (Harlan). Batf<sup>ΔZ/KI</sup> mice were mated to produce Batf<sup>ΔZ/KI</sup> mice which were crossed to EIIaCre mice (JAX). Batf<sup>ΔZ</sup> mice were backcrossed to C57BL/6 mice four to six times and were mated to generate Batf<sup>ΔZ/ΔZ</sup> and littermate control Batf<sup>ΔZ/ΔZ</sup> and Batf<sup>−/−</sup> mice that were used for experimentation at 7–12 wk of age. The genotyping primers for Batf<sup>ΔZ</sup> are forward, 5′-GCTTGTCTCTCACTAGT-3′, and reverse, 5′-CTGTTAGTAGCTTGCTAC-3′. All mice used in this study were maintained in a specific pathogen-free animal facility according to institutional guidelines. All animal protocols were reviewed and approved by the Purdue University Animal Care and Use Committee.

**DNA blot hybridization.** 20 µg DNA, isolated from tail tips by phenol/chloroform extraction, was digested with SpeI and resolved by 0.8% agarose gel electrophoresis. DNA was transferred to Zeta Probe membrane (Bio-Rad Laboratories), cross-linked, and probed using the Batf cDNA as previously described (Williams et al., 2001).

**Immunoblot.** Protein was isolated from stimulated splenocytes using RIPA buffer supplemented with protease inhibitors. Immunoblots to detect
Figure 6. Batf^ΔZ/ΔZ B cells do not undergo CSR. (A) B cells purified from Batf^+/+ and Batf^ΔZ/ΔZ mice were cultured in media, or media supplemented with 20 µg/ml LPS, with or without 20 ng/ml IL-4. After 24 h, DNA synthesis was quantified by BrdU labeling for 16 h. Shown is mean BrdU incorporation, relative to Batf^+/+ or Batf^ΔZ/ΔZ cells in media (set to 1.0), from three experiments (n = 3) performed in triplicate. Error bars indicate SE. (B) B cells cultured as in A were assayed for surface Ig expression by flow cytometry (top) and for secreted Ig by ELISA (bottom). The mean and SE were calculated from three experiments (n = 3). N, not detected. (C) RNA from cells in B was assayed for the indicated transcripts using qPCR. Data are averaged from three experiments (n = 3) performed in triplicate and expressed relative to the Batf^+/+ media control (set to 1.0). Error bars indicate SE. N, not detected. (D) Sera from Batf^+/+ and Batf^ΔZ/ΔZ mice immunized with TNP-LPS or PBS were analyzed by ELISA for TNP-specific IgG1. Mean results from three mice per group (n = 3) are plotted. Error bars indicate SE. (E) Spleen sections from mice in D (n = 3 for each genotype) were stained as in Fig. 3 C to detect IgG1- and IgG2c-producing cells. Representative images are shown (20x). f, follicle. Bars, 50 µm.
Cell culture. Splenocytes were prepared and CD4+ T and resting B cells isolated using CD4 (L3T4) and CD43 magnetic bead separation, respectively (Miltenyi Biotec). Cells were cultured as previously described (Sayner et al., 1988; Williams et al., 2003). Stimulations and in vitro skewing conditions are detailed in figure legends. B cell proliferation was measured using a BrdU cell proliferation kit (Milipore).

Analysis of RNA. Semi-qPCR was performed as previously described (Thornton et al., 2006) and qPCR was performed with SYBR green (Roche) and a real-time PCR system (7300; Applied Biosystems). Primers are listed in figure legends or Table S1. Results were normalized to Hprt or β-actin expression. ΔCt values were used to calculate relative expression of each mRNA.

ELISA. Blood, collected by cardiac puncture, was allowed to clot at room temperature in a Microtainer Serum Separator tube (BD). Serum was isolated by centrifugation. ELISA for IgG2c and IgE was performed using OptiElisa kits (BD). ELISA for IgG1, IgA, and IgM used mAb indicated in Table S2. Serum was diluted in Assay Diluent (BD) and applied to Ab-coated 96-well MaxiSorp plates (Nunc), and reactions were visualized with streptavidin-HRP and TMB substrate reagent (BD). ELISA plates and protocols for detecting sRBC-specific IgM and IgG were obtained from Life Technologies and IgG1 was obtained from Southern Biotech. ELISA to detect TNP-LPS–specific IgG1 used plates coated with 10 µg/ml TNP-BSA (Biosearch Technologies).

In vivo antigen stimulation and IHC. For T cell–dependent response, mice were injected i.p. with 5 × 10^7 sRBC in 200 µl PBS or PBS alone (mock). On day 8, sera were isolated for ELISA and spleen tissue was processed for IHC as previously described (Zhu et al., 2007). For T cell–independent response, mice were injected with 30 µg TNP-LPS (Sigma–Aldrich) or an equal volume of PBS. On day 8, sera were isolated for ELISA and spleen tissue was processed for IHC. GCs were detected using biotinylated PNA (1:100) and Texas Red conjugated streptavidin-HRP and TMB substrate reagent (BD). ELISA plates and protocols for detecting rRBC-specific IgM and IgG were obtained from Life Diagnostics and IgG1 was obtained from Southern Biotech. ELISA to detect TNP-LPS–specific IgG1 used plates coated with 10 µg/ml TNP-BSA (Biosearch Technologies).

Adoptive transfer and immunization. On day 9, 5 × 10^5 CD4+ T cells from Batf−/− or Batf+/+ mice were injected i.v. into T cells–deficient mice (B6.129P2-Tg(lmEB2/6Smt; Tin[lmEB2/6SmtJ]; JAX) and animals immunized i.p. with 5 × 10^7 sRBC in 100 µl PBS. Mice were sacrificed on day 8 for the analysis of T cell subsets and Ig production.

Chemotaxis assay. Chemotaxis assays were performed and analyzed as described previously (Lim et al., 2004). In brief, 5 × 10^4 lymphocytes from PP were added to the upper chamber of Transwell inserts (Corning) and allowed to migrate to media in a lower chamber containing 2.5 µg/ml rmCXCL13. After 3 h, cells were collected and analyzed by flow cytometry.

Online supplemental material. Fig. S1 shows the expression of the mouse Batf gene in Batf−/−, Batf+/+, and Batf+/+ mice. Fig. S2 compares the thymic T cell profile, the T reg cell profile, and the in vitro T reg, Th1, and Th17 differentiation profiles of Batf−/− and Batf+/+ mice. Table S1 contains sequences of the oligonucleotide primers used in this study. Table S2 provides information on the antibodies used in this study. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20091548/DC1.

The authors thank J.P. Robinson, K. Ragheb, C. Holdman, and the Purdue University Cytometry Laboratory for assistance with flow cytometry and S. Konieczny, J. Hallett, A. Kaufman, and the Transgenic Mouse Core Facility of the Purdue University Center for Cancer Research for the generation of BatfKI mice. Special thanks are extended to K. Williams and A. Zullo, whose Ph.D. thesis research provided a foundation for this work.

This study was supported by National Institutes of Health grants CA782464 and CA114381 (E. Taparowsky) and National Institutes of Health grant AI074745 (C.H. Kim). Predoctoral student support was provided by National Institutes of Health grant T32 GM08298 (K.L. Jordan-Williams and M.R. Logan) and by a Career Development Award from the Indiana CTSI (National Institutes of Health grant 5T1 RR025759 to M.R. Logan).

The authors have no conflicting financial interests.

Submitted: 17 July 2009
Accepted: 23 March 2010

REFERENCES

Amoutzias, G.D., E. Bornberg-Bauer, S.G. Oliver, and D.L. Robertson. 2006. Reduction/oxidation-phosphorylation control of DNA binding in the bZIP dimerization network. BMC Genomics. 7:107–117. doi:10.1186/1471-2164-7-107

Arnonheim, A., E. Zandi, H. Hennemann, S.J. Elledge, and M. Karin. 1997. Isolation of an AP-1 repressor by a novel method for detecting protein-protein interactions. Mol. Cell. Biol. 17:3094–3102.

Dorsey, M.J., H.J. Tae, K.G. Sollenberger, N.T. Maccarenhas, L.M. Johansen, and E.J. Taparowsky. 1998. B-ATF: a novel human bZIP protein that associates with members of the AP-1 transcription factor family. Oncogene. 11:2255–2266.

Echlin, D.R., H.J. Tae, N. Mitin, and E.J. Taparowsky. 2000. B-ATF functions as a negative regulator of AP-1-mediated transcription and blocks cellular transformation by Ras and Fox. Oncogene. 19:1752–1763. doi:10.1038/sj.onc.1203491

Eferl, R., and E.F. Wagner. 2003. AP-1: a double-edged sword in tumorigenesis. Nat. Rev. Cancer. 3:859–868. doi:10.1038/nrc1209

Fairfax, K.A., A. Kallies, S.L. Nutt, and D.M. Tarlinton. 2008. Plasma cell differentiation: from B-cell subsets to long-term survival niches. Semin. Immunol. 20:49–58. doi:10.1016/j.seminimmunol.2007.12.002

Hess, J., P. Angel, and M. Schoppk-Kistner. 2004. AP-1 subunits: quarrel and harmony among siblings. J. Cell Sci. 117:5965–5973. doi:10.1242/jcs.015889

Hildner, K., B.T. Edelson, W.E. Purtha, M. Diamond, H. Matsushita, M. Koyama, B. Calderon, B.U. Schraml, E.R. Unarue, M.S. Diamond, et al. 2008. Batf3 deficiency reveals a critical role for CD38alpha+ dendritic cells in cytotoxic T cell immunity. Science. 322:1097–1100. doi:10.1126/science.1146206

Honjo, T., M. Muramatsu, and S. Fagarasan. 2004. AID: how does it aid EBNA2 and activated Notch induce expression of BATF. J. Immunol. 165:860–866.

Johansen, L.M., C.D. Depmann, K.D. Erickson, W.F. Coffin III, T.M. Thornton, S.E. Humphrey, J.M. Martin, and E.J. Taparowsky. 2003. EBNA2 and activated Notch induce expression of BATF. J. Virol. 77:6029–6040. doi:10.1128/JVI.77.10.6029-6040.2003

Jung, M., R. Sabat, J. Krätzschmar, H. Seidel, K. Wolk, C. Schönbein, S. Schütt, M. Friedrich, W.D. Döcke, K. Asadullah, et al. 2004. Expression profiling of IL-10-regulated genes in human monocytes and...
