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Regulation of IFN-γ Production by B Effector 1 Cells: Essential Roles for T-bet and the IFN-γ Receptor[1]

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This manuscript systematically identifies the molecular mechanisms that regulate the ability of B cells to produce the critical type 1 cytokine, IFN-γ. B cells produce IFN-γ in response to IL-12 and IL-18 and when primed by Th1 cells. We show that development of IFN-γ-producing B cells by either Th1 cells or IL-12/IL-18 is absolutely dependent on expression of the IFN-γR and the T-box transcription factor, T-bet. Interestingly, although T-bet up-regulation in developing B effector 1 (Be1) cells is controlled by IFN-γR-mediated signals, STAT1-deficient B cells up-regulate T-bet and produce IFN-γ, indicating that additional transcriptional activators must be coupled to the IFN-γR in B cells. Finally, we show that although IL-12/IL-18 or IFN-γ-producing Th1 cells are required to initiate transcription of the IFN-γ gene in B cells, sustained expression of IFN-γ and T-bet by B cells is dependent on an IFN-γ/IFN-γR/T-bet autocrine feedback loop. These findings have significant implications, because they suggest that IFN-γ-producing B cells not only amplify Th1 responses, but also imprint a type 1 phenotype on B cells themselves. In the case of immune responses to bacterial or viral pathogens, this B cell-driven autocrine feedback loop is likely to be beneficial; however, in the case of B cell responses to autoantigens, it may result in amplification of the autoimmune loop and increased pathology. ***The Journal of Immunology,*** **2005, 174: 6781–6790.**

It is now well established that B cells produce cytokines in response to a diverse array of stimuli, including microbial products, Ag, and T cells (1, 2). Furthermore, naive B cells can be induced to differentiate into discrete B cell effector subsets that will produce differential arrays of cytokines upon restimulation, and these cytokine-producing effector B cells can modulate T cell responses (3). Cytokine-producing B cell effectors have been identified in the draining lymph nodes and spleens of autoimmune mice (4–6) and mice infected with pathogens that induce strong type 1 or type 2 immunity (3, 7, 8). Importantly, cytokine-producing B cells play a critical protective role in several autoimmune disease models, including inflammatory bowel disease (4), experimental autoimmune encephalomyelitis (6), and collagen-induced arthritis (5). Therefore, understanding the molecular mechanisms that control cytokine production by B cells is becoming increasingly important.

One of the cytokines produced in large quantities by activated B cells is IFN-γ. Although IFN-γ-producing B cells have been identified in mice infected with pathogens such as *Toxoplasma gondii* and *Borrelia burgdorferi* (3, 7), the molecular mechanisms that regulate IFN-γ production by B cells are not known. It has been previously shown that B cells produce IFN-γ in response to Ag (9) and to cytokines such as IL-12 and IL-18 (10, 11). In addition, B cells cultured in the presence of polarized Th1 cells and Ag develop into high rate IFN-γ-producing B effector 1 (Be1)[3] cells that are capable of promoting the differentiation of naive T cells into Th1 effectors (3). Thus, the data suggest that some combination of BCR signals, T cell help, cytokines, and pathogen-derived signals is necessary to induce the differentiation of naive B cells into IFN-γ-producing effectors.

Similar factors are reported to influence the differentiation of naive T cells into IFN-γ-producing Th1 effectors (12). However, the cytokine environment in which naive T cells are primed is believed to play the most critical role in regulating commitment to the Th1 lineage (12–16). Although there is still some debate as to which cytokine-producing pathways are most important for initiating, amplifying, and sustaining the Th1 differentiation process, it is clear that IFN-γ, IL-12, and IL-27 are important (17–21). In the current model, binding of IFN-γ to the IFN-γR on naive T cells induces the recruitment, activation, and nuclear translocation of STAT1 (22, 23). STAT1 up-regulates the expression of the transcription factor T-bet (24, 25), a recently described member of the T-box family of transcription factors (26). Likewise, activation of naive T cells via the IL-27R and STAT1 is reported to up-regulate T-bet expression (27). T-bet, in turn, induces chromatin remodeling of the IFN-γ gene and thereby promotes IFN-γ gene transcription (28–30). T-bet also augments the expression of the IL-12Rβ2 subunit, allowing the T cells to become fully responsive to IL-12 (24, 30). Activation of IL-12R leads to the recruitment and translocation of STAT4 to the nucleus, where it is believed to interact with the transcriptional coactivator CREB binding protein to promote IFN-γ expression (31, 32). Induction of IFN-γ gene transcription by T-bet and STAT4/CBP leads to IFN-γ production and presumably the initiation of an IFN-γ-driven positive feedback loop that drives the full commitment of T cells to the Th1 lineage (12).

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[3] Abbreviations used in this paper: Be1, B effector 1; HA, hemagglutinin; HEL, hen egg lysozyme; ICCS, intracellular cytokine staining; PCCF, pigeon cytochrome *c* fragment; qPCR, quantitative PCR; Tg, transgenic; WT, wild type; BAEF, B cell-activating factor of the TNF family; RPL32, ribosomal protein L32.

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Similar to CD4 T cells, naive B cells express IFN-γR, IL-12R, STAT1, STAT4, and T-bet. Using an in vitro T cell- and Ag-driven cognate model of Be1 development, we have now identified the molecular mechanisms that control IFN-γ production by B cells. We demonstrate that IFN-γ production by B cells activated with Th1 cells and Ag is dependent on IFN-γR and T-bet, but can occur in the absence of STAT1 or BCR signals. Likewise, IFN-γ production by B cells activated in the presence of IL-12 is T-bet and IFN-γR dependent. Furthermore, autocrine production of IFN-γ by B cells activated with either Th1 cells or IL-12 is required to maintain IFN-γ gene transcription. Thus, IFN-γ-producing B cell effectors not only promote Th1 development, but are also able to sustain their own development, suggesting that IFN-γ-producing B cells may play an important role in amplifying T cell-dependent type 1 responses.

Materials and Methods

Mice

The following strains of mice were bred at Trudeau Institute: B10.BR, BALB/c, C57BL/6J, IFN-γR-deficient mice (*IFN-γR*$^{-/-}$ backcrossed to C57BL/6), IFN-γ-deficient mice (*IFN-γ*$^{-/-}$ backcrossed to B10.BR), hen egg lysozyme (HEL)-specific BCR transgenic (Tg) mice (MD4 mice backcrossed to B10.BR), OVA-specific CD4 TCR Tg mice (OT-II mice backcrossed to C57BL/6J), hemagglutinin (HA)-specific CD4 TCR Tg mice (HNT mice backcrossed to BALB/c), pigeon cytochrome *c* fragment (PCCF)-specific CD4 TCR Tg mice on the B10.BR background (AND B10.BR mice), and AND B10.BR mice crossed to *IFN-γ*$^{-/-}$ B10.BR mice (AND × *IFN-γ*$^{-/-}$ B10.BR mice). *STAT4*$^{-/-}$ mice were purchased from The Jackson Laboratory. *STAT1*$^{-/-}$ mice and control 129S6/SvEv mice were purchased from Taconic Farms. *T-bet*$^{-/-}$ mice on the C57BL/6 background were bred at Washington University (St. Louis, MO). All procedures involving animals were approved by the Trudeau Institute institutional animal care and use committee and were conducted according to the principles outlined by the National Research Council.

Reagents

Recombinant cytokines were obtained from the following sources: human IL-2 and mouse IL-18, R&D Systems; B cell-activating factor of the TNF family (BAFF), BioSource International; IL-12, Dr. S. Wolf (Genetics Institute, Cambridge, MA); and IFN-γ, purified from IFN-γ-transfected cells. Abs were obtained from the following sources: anti-IL-12 (clone C17.8) and anti-CD40 Ab (clone 1C10), Bioexpress; goat anti-mouse IgM, ICN Biomedicals; and anti-IL-4 (clone 11B11) and anti-IFN-γ (clone XMG1.2), National Cancer Institute. All fluorochrome-labeled Ab and purified anti-CD28 (clone 37.51) were purchased from BD Biosciences. $PCCF_{88-104}$, $OVA_{323-339}$, and HA_{26-138} were purchased from New England Peptide.

Purification of naive and effector cells

Naive or effector T and B cells were incubated with either anti-CD4 or anti-CD19-coupled magnetic beads and then purified by MACS (Miltenyi Biotec). Cell purities, as determined by FACS analysis of live cells, were routinely 90–95% for naive B and T cells and >99% for Be1 cells.

Generation of Th1 cells

Purified, naive splenic CD4 T cells were cultured in complete tissue culture medium containing IL-2 (100 ng/ml), IL-12 (2 ng/ml), and anti-IL-4 (10 μg/ml) with anti-CD3 (10 μg/ml) and anti-CD28 (5 μg/ml). In some experiments, IFN-γ (20 ng/ml) was also added to the cultures.

T cell-dependent generation of Be1 cells

Purified naive splenic B cells were cultured with mitomycin C-treated syngeneic, Tg Th1 effector cells (1/1). Cultures were supplemented with 5 μM peptide, (PCCF, OVA, or HA) and either HEL (10 μg/ml) or anti-IgM (25 μg/ml) depending on the haplotype of the B and T cells used. IL-2 (50 ng/ml) was added on day 2. At 24–96 h, cells were harvested and purified by positive selection (>99% purity) using anti-CD19 magnetic beads. Cytokine production by effector B cells was, unless stated otherwise, in response to an overnight (10-h) restimulation with PMA (5 ng/ml) and Ca^{2+} ionophore (1.25 μM).

T cell-independent stimulation of B cells

Purified, naive splenic B cells were cultured with LPS (25 μg/ml), anti-IgM (25 μg/ml), or anti-CD40 (15 μg/ml) in the presence of IL-12 (2 ng/ml) and IL-18 (20 ng/ml). Supernatants were collected at 72 h for analysis of cytokine production. Alternatively, purified naive B cells were cultured with anti-IgM, anti-CD40, and BAFF (20 ng/ml) in the presence of cytokines and anti-cytokine Ab as indicated. On day 4, the B cells were repurified (>99% $CD19^+$) and restimulated.

Detection of secreted cytokines

Cytokines were measured by ELISA using anti-cytokine Ab pairs (BD Biosciences) or by Luminex-based multiplex cytometric bead array using Beadlyte multicytokine beads (Upstate Biotechnology).

CFSE labeling

The fluorescent dye CFSE (Molecular Probes) was added (0.2 μM) to purified naive B cells for 15 min at 37°C.

PCR

Total RNA was extracted from cell pellets using the RNeasy kit (Qiagen). RNA was quantified and reverse transcribed using SuperScript II (Invitrogen Life Technologies). cDNA was normalized to 10 ng/μl. Final concentrations of all primers and probes were 100 nM in a 25-μl reaction volume that contained 50 ng of cDNA and diluted 2× Mastermix (Applied Biosystems). For quantitative PCR (qPCR), the following conditions were used: one cycle at 50°C for 2 min, one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Semiquantitative PCR samples were multiplexed with IFN-γ-specific primers and ribosomal protein L32 (RPL32)-specific primers. Multiplexed samples were amplified using the following PCR conditions: one cycle at 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, 54°C for 30 s, and 72°C for 1 min, ending with one cycle at 72°C for 5 min. The PCR products were run on a 2% agarose gel. The IFN-γ PCR yielded a 223-bp product, and RPL32 PCR yielded a 261-bp product.

The mouse IFN-γ-specific qPCR primers were purchased from Applied Biosystems. All other qPCR primer/probe sets were designed and tested by the Trudeau Institute Molecular Biology Core Facility. The IFN-γ- and RPL32-specific primers used for the semiquantitative multiplex assay were synthesized by Genosys (Sigma-Aldrich). The IFN-γ forward primer begins 100 bp upstream of the initial methionine codon, and the reverse primer spans the exon 1/exon 2 boundary from +95 to +123 bp. The sequences of the noncommercial primers and probes are listed below: T-bet: forward, 5′-CCTGTTGTGGTCCAAGTTCAAC-3′; reverse, 5′-CACAAACATCCTGTAATGGCTTGT-3′; probe, 5′-ATCATCACTAAGCAAGGACGGCGAATGTTCC-3′; Gata-3: forward, 5′-GCAGGTCACACGCCTCCT-3′; reverse, 5′-GATTCCTTGGCGCTCAGAGA-3′; probe, 5′-CCTTGCTACTCAGGATCGGAAGAGCAACC-3′; IFN-γ: forward, 5′-CCATCGGCTGACCTAGAGAAGAC-3′; reverse, 5′-GCCACTTGAGTTAAAATAGTTATTCAGAC-3′; and RPL32: forward, 5′-GGCGGAAACCCAGAGGCATTGACA-3′; reverse, 5′-GTAGCCTGGCGTTGGGATTGGTGACTC-3′.

Results

IFN-γ production by effector B cells is regulated by genetic factors and T cell-derived signals

We previously showed that we can generate highly enriched populations of IFN-γ-producing Be1 cells by culturing naive B cells with Th1 effectors and Ag in vitro (3). To determine the kinetics of IFN-γ mRNA induction in the developing Be1 cells, we purified naive splenic B cells from HEL-specific BCR Tg mice and cultured the B cells with mitomycin C-treated PCCF-specific TCR Tg Th1 day 4 effectors. We added PCCF peptide and HEL protein to the cultures to facilitate cognate interactions between the B and T cells and to induce TCR and BCR signaling, but did not add any exogenous cytokines or anti-cytokine Abs. At 24-h intervals, we purified the B cells by positive selection and prepared mRNA. We also prepared mRNA from purified naive B cells (0 h) and from day 3 Be1 cells that were restimulated. We then determined IFN-γ levels in the B cells by qPCR. As shown in Fig. 1*A*, we detected IFN-γ mRNA at low levels in naive B cells and, within 24 h of activation, we found that IFN-γ mRNA levels increased ~100-fold. IFN-γ mRNA levels continued to increase in the Be1 cells

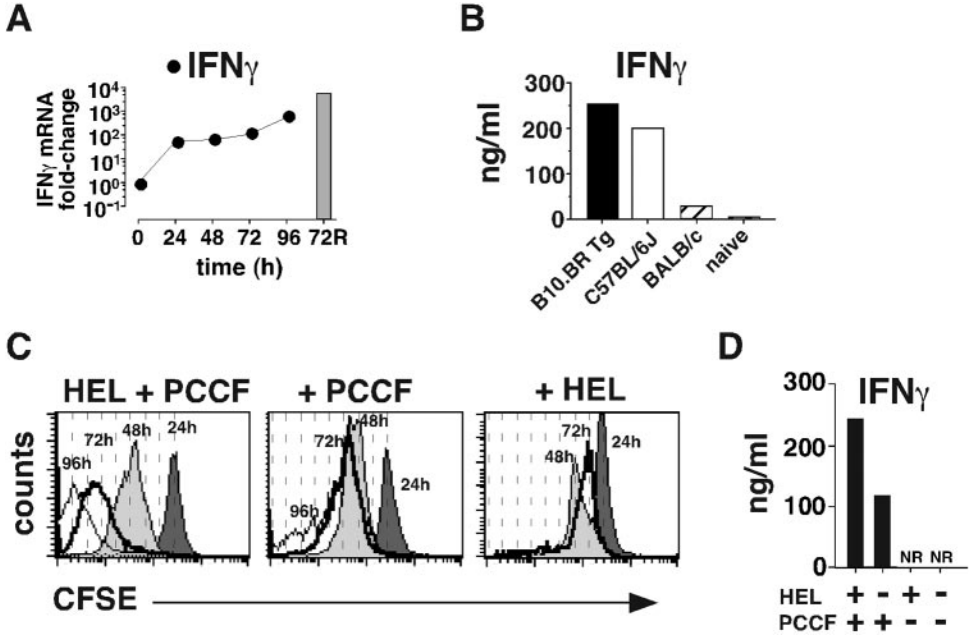

FIGURE 1. IFN-γ production by Be1 cells is regulated by genetic factors and T cell-derived signals. *A*, Purified B cells from HEL-specific BCR Tg mice were cultured with PCCF-specific TCR Tg Th1 cells and Ag (HEL + PCCF peptide). At time zero and at 24-h intervals, mRNA was prepared from repurified B cells (>99% $CD19^+$). At 72 h, repurified B cells were restimulated for 8 h, and mRNA was prepared (72R, ▨). IFN-γ mRNA expression levels were determined by qPCR and normalized to GAPDH levels. The data are presented as the fold difference between naive B cells, which expressed detectable levels of IFN-γ transcripts, and all other samples. *B*, Be1 cells were generated by culturing naive B cells from different strains of mice with TCR Tg Th1 cells in the presence of Ag (HEL or anti-IgM and appropriate peptides). At 96 h, repurified Be1 cells (>99% $CD19^+$) were restimulated for 10 h, and IFN-γ levels were determined by ELISA. The amount of IFN-γ produced by naive B cells stimulated with PMA plus Ca^{2+} ionophore is shown for comparison. In all cases, the amount of IFN-γ produced by stimulated naive B cells was <5 ng/ml. *C* and *D*, CFSE-labeled, naive, HEL-specific B cells were cultured with PCCF-specific Th1 effectors in the presence of HEL plus PCCF, PCCF alone, or HEL alone for 24–96 h. *C*, At 24-h intervals, cells were harvested, and the expression of CFSE on $CD19^+$ cells was analyzed by flow cytometry. *D*, At 96 h, B cells were purified (>99% $CD19^+$) and restimulated for 10 h, and IFN-γ levels were determined. NR, no cells recovered. The data are representative of two or more independent experiments.

over the course of the 96-h culture period and were further elevated after restimulation (Fig. 1*A*). Thus, naive B cells constitutively express IFN-γ mRNA and can be induced to rapidly upregulate IFN-γ mRNA levels upon culture with Th1 cells and Ag.

To identify the factors that control IFN-γ expression in developing Be1 cells, we first determined whether IFN-γ production by Be1 cells is influenced by the genetic background of the B cells. We therefore isolated naive splenic B cells from C57BL/6J mice ($H\text{-}2^b$), BALB/c mice ($H\text{-}2^d$), or B10.BR HEL-specific BCR Tg mice ($H\text{-}2^k$) and then cultured the B cells for 4 days with syngeneic TCR Tg effector Th1 cells in the presence of anti-IgM (non-Tg B cell cultures), HEL (HEL-specific Tg B cell cultures), and class II-restricted peptides that could be presented by B cells to Tg Th1 cells. After 4 days, we purified Be1 cells from each of the cultures, then restimulated B cells with PMA and Ca^{2+} ionophore. B10.BR BCR Tg Be1 cells and C57BL/6J non-Tg Be1 cells secreted high levels of IFN-γ (~200 ng/ml; Fig. 1*B*). In contrast, Be1 cells derived from BALB/c mice produced ~10-fold less IFN-γ (Fig. 1*B*). Although the amount of IFN-γ produced by BALB/c Be1 cells was reduced compared with that produced by B10.BR or C57BL/6J Be1 cells, the restimulated BALB/c Be1 cells still produced significantly more IFN-γ than stimulated naive B cells (Fig. 1*B*). Thus, although all splenic B cells are capable of producing IFN-γ in the presence of Th1 cells and Ag, the genetic background of B cells influences the magnitude of IFN-γ produced by effector B cells.

We have previously shown that signaling through the BCR is not necessary for the development of IFN-γ-producing Be1 cells (3). To assess the role of T cell-derived signals in the development, survival, or expansion of Be1 cells, we purified naive HEL-specific Tg B cells, labeled the cells with CFSE, and cultured them with PCCF-specific Th1 effector cells in the presence or the absence of PCCF peptide and/or HEL protein. At 24-h intervals we harvested the cells, stained them with anti-CD19, and analyzed them by flow cytometry (Fig. 1*C*). After 96 h we repurified the surviving B cells and restimulated equivalent numbers of these cells with PMA and Ca^{2+} ionophore to measure IFN-γ production (Fig. 1*D*). Naive B cells activated in the presence of Th1 cells, HEL, and PCCF underwent division in a relatively synchronous fashion, as evidenced by the uniform levels of CFSE expressed within the entire dividing B cell population at each time point analyzed (Fig. 1*C*). Within 96 h, CFSE expression levels had declined to background levels in all B cells stimulated with Th1 cells and PCCF plus HEL, indicating that all surviving B cells had divided at least seven times (Fig. 1*C*). As expected, these B cells made large amounts of IFN-γ after restimulation (Fig. 1*D*). In contrast, B cells that were cultured in the presence of Th1 cells and PCCF (no BCR signal) divided less efficiently, and by 96 h only 15% of the B cells had undergone five or more rounds of division (Fig. 1*C*). Despite the limited proliferation of these B cells, they still produced large quantities of IFN-γ (>100 ng/ml; Fig. 1*D*). Finally, B cells that were cultured in the presence of Th1 cells and HEL alone (no B/T cognate interaction) underwent only a limited number of cell divisions (less than two), and by 72 h there were no viable B cells remaining in the cultures (Fig. 1, *C* and *D*). Together, these data indicate that a cognate interaction between B and T cells is necessary for the survival and expansion of Be1 cells, and although a BCR-specific signal facilitates expansion of the developing Be1 cells, neither the survival of these cells nor their ability to produce IFN-γ is dependent on a BCR signal.

IFN-γR expression by B cells is required for the development of IFN-γ-producing Be1 cells

The data in Fig. 1 suggested that the differentiation of naive B cells into IFN-γ-secreting Be1 cells is facilitated by signals delivered by T cells to peptide-presenting B cells. Because Th1 effectors rapidly produce IFN-γ in response to APCs (33), and IFN-γ is known to regulate the differentiation of naive T cells to Th1 effectors by an IFN-γR-dependent mechanism (34), we considered the possibility that Be1 cell development might also be regulated by an IFN-γR-dependent signal. To test this hypothesis, we cultured naive B cells purified from either wild-type (WT) C57BL/6J mice or *IFN-γR*$^{-/-}$ C57BL/6J mice with OVA-specific TCR Tg Th1 effector cells in the presence of class II-restricted OVA peptide and anti-IgM Ab. We purified B cells on day 4, restimulated the cells with PMA and Ca^{2+} ionophore, and then assayed for IFN-γ. Interestingly, IFN-γ production by IFN-γR-deficient B cells was reduced by 95% compared with that by WT Be1 cells and, although not completely absent (~8 ng/ml; Fig. 2*A*), was only slightly elevated over that normally seen in stimulated naive B cells (see Fig. 1*B*). The reduction in IFN-γ production by IFN-γR-deficient Be1 cells was not due to an inability of these B cells to respond to BCR and T cell-dependent signals, because the IFN-γR-deficient Be1 cells expanded more vigorously in culture (data not shown) and produced significantly more IL-2 than WT Be1 cells (Fig. 2*A*).

STAT1-independent production of IFN-γ by Be1 cells

IFN-γ production in CD4 T cells is controlled by the IFN-γR/STAT1 signaling pathway (12). Because we found that IFN-γ production by Be1 cells required the expression of IFN-γR (Fig. 2*A*), we predicted that IFN-γ production by B cells would also be STAT1 dependent. To test this hypothesis, we cultured purified naive B cells from WT or STAT1-deficient mice with Th1 cells and Ag (anti-IgM and OVA peptide). After 4 days, we repurified the B cells, stimulated the cells, and measured IFN-γ production. Interestingly, STAT1-deficient B cells produced significant amounts of IFN-γ. Indeed, in most experiments STAT1-deficient B cells produced IFN-γ at levels equivalent to or higher than those produced by WT B cells (Fig. 2*B*). In some experiments the amount of IFN-γ produced by STAT1-deficient Be1 cells was reduced ~2-fold compared with that produced by WT B cells (data not shown). However, in all experiments IFN-γ-producing B cells, as measured by intracellular cytokine staining (ICCS), were easily detected in the restimulated STAT1-deficient B cells (Fig. 2*C*). Interestingly, STAT1-deficient Be1 cells, like IFN-γR-deficient B cells, expanded more efficiently in culture (data not shown) and produced more IL-2 than did WT Be1 cells (Fig. 2*B*). Together, these data show that although the differentiation of naive B cells into an IFN-γ-producing Be1 cell is strictly dependent upon signals generated through the IFN-γR, signaling through STAT1 is not required for Be1 development. However, IFN-γR/STAT1-dependent signals appear to negatively regulate B cell expansion and IL-2 production.

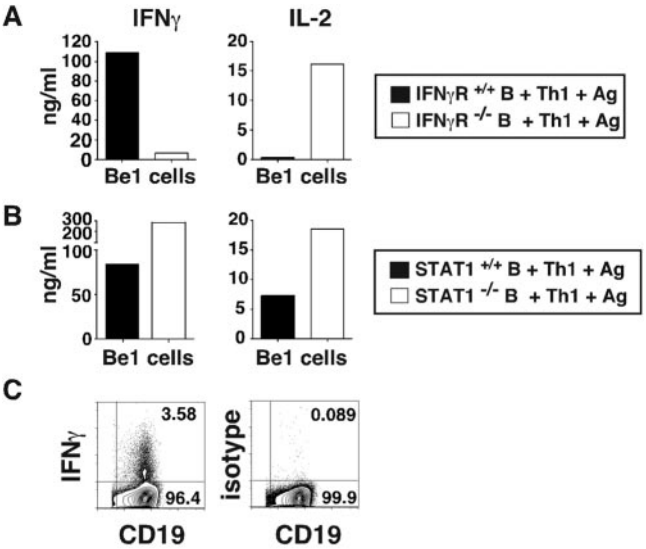

FIGURE 2. Be1 development is dependent on IFN-γR signals, but not STAT1. *A*, Purified naive B cells isolated from C57BL/6J mice or IFN-γR-deficient mice were cultured with OVA-specific Th1 effectors and Ag (anti-IgM + OVA peptide) for 96 h. B cells were purified (>99% $CD19^+$) and restimulated for 10 h, and IFN-γ and IL-2 levels were determined. *B* and *C*, Purified naive B cells from WT 129S6/SvEv mice or STAT1-deficient mice were cultured with OVA-specific Th1 cells and Ag (anti-IgM + OVA peptide) for 96 h. Be1 cells were harvested, purified (>99% $CD19^+$), and restimulated for 10 h, and IFN-γ production was measured by ELISA (*B*) or in STAT1-deficient B cells by ICCS (*C*). Data are representative of three independent experiments.

Signaling through IFN-γR is required for up-regulation of T-bet in developing Be1 cells

IFN-γR-mediated Th1 development is controlled by the transcription factor T-bet in CD4 T cells (25, 26, 29), whereas Th2 development is regulated by GATA-3 (35, 36). Although T-bet expression by IFN-γ-producing B cells has previously been reported (26, 37), the factors that control T-bet expression within the B cell lineage are not characterized. Therefore, we examined the kinetics of T-bet and GATA-3 expression in naive and activated Be1 cells by qPCR. Both T-bet and GATA-3 transcripts were detected at low levels in naive, HEL-specific BCR Tg B cells (Fig. 3*A*). T-bet levels in the B cells increased ~20-fold within 24 h of activation with Th1 cells, PCCF peptide, and HEL and remained elevated throughout the 96 h of culture (Fig. 3*A*). In contrast, the expression of GATA-3 mRNA in B cells activated with Th1 cells and Ag decreased rapidly after initiation of the cultures and remained depressed throughout the time course (Fig. 3*A*).

Next we examined whether induction of T-bet mRNA in B cells is mediated through an IFN-γR-dependent pathway. We prepared mRNA from naive and activated Be1 cells generated from WT and *IFN-γR*$^{-/-}$ B cells and then determined T-bet expression levels by qPCR. Although T-bet was expressed in naive B cells isolated from IFN-γR-deficient mice, the expression level was very low and was reduced at least 15-fold compared with that in WT naive B cells (Fig. 3*B*). Furthermore, in contrast to what we observed with *IFN-γR*$^{+/+}$ B cells, the expression of T-bet in IFN-γR-deficient B cells did not increase after activation with Th1 cells and Ag (Fig. 3*B*). As expected, the expression of GATA-3 mRNA was equivalent in naive WT and IFN-γR-deficient B cells (Fig. 3*C*). Furthermore, after activation, GATA-3 levels dropped below the threshold of detection by qPCR in WT and *IFN-γR*$^{-/-}$ B cells (Fig. 3*C*), indicating that IFN-γR-deficient B cells did not simply switch and differentiate into GATA-3-expressing, IL-4-producing cells. Taken together, the data show that the expression of IFN-γR by B cells is required for up-regulation of T-bet in response to Th1 cells and Ag.

Given that STAT1 is not required for the development of Be1 cells (Fig. 2*B*), we predicted that T-bet would be up-regulated in STAT1-deficient B cells. To test this hypothesis, we cultured WT and *STAT1*$^{-/-}$ B cells in the presence of Th1 cells, anti-IgM, and peptide. At 0, 24, and 96 h, we repurified the B cells and measured T-bet levels by qPCR. As shown in Fig. 3*D*, T-bet mRNA was up-regulated in both *STAT1*$^{+/+}$ and *STAT1*$^{-/-}$ B cells. Although

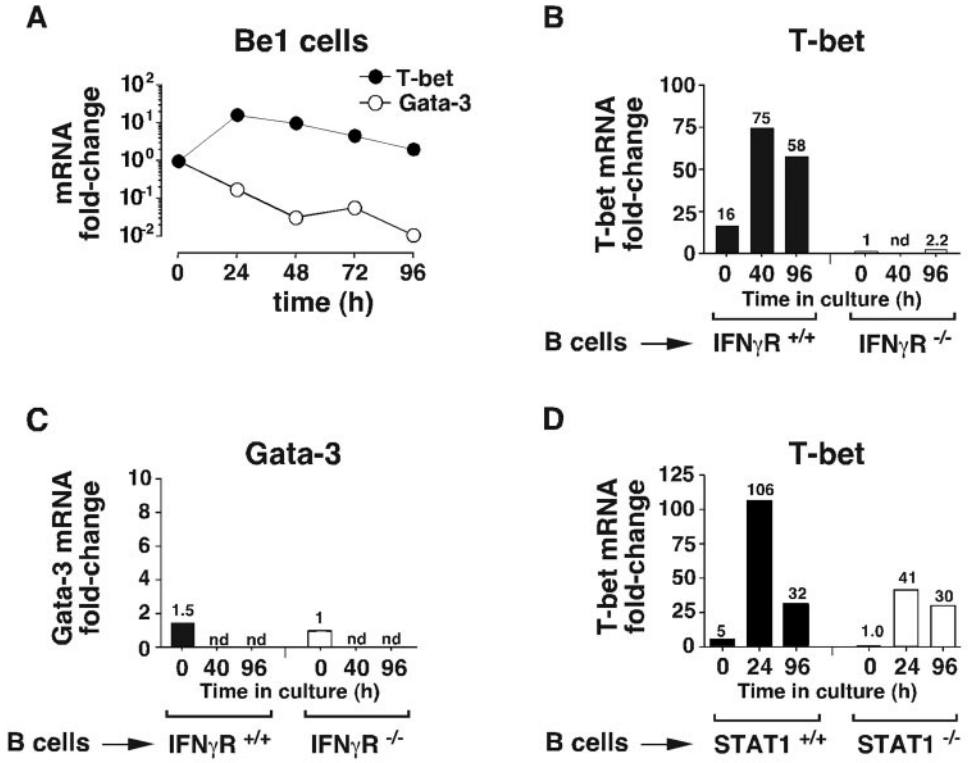

FIGURE 3. IFN-γR-dependent, STAT1-independent up-regulation of T-bet in developing Be1 cells. *A*, Purified naive HEL-specific B cells were cultured with PCCF-specific Th1 cells and Ag (HEL + PCCF). At 24-h intervals, Be1 cells were purified, and mRNA was isolated. T-bet and GATA-3 mRNA levels were determined by qPCR and normalized to GAPDH levels. The data are presented as the fold difference between naive B cells, which expressed detectable levels of T-bet and GATA-3 transcripts, and all other samples. *B* and *C*, Purified naive B cells isolated from C57BL/6J mice or IFN-γR-deficient mice were cultured with OVA-specific Th1 cells and Ag (anti-IgM + OVA peptide). B cells were repurified at the indicated times, and mRNA was isolated. T-bet (*B*) and GATA-3 (*C*) mRNA levels were determined by qPCR. The data are presented as the fold difference between naive *IFN-γR*$^{-/-}$ B cells, which expressed very low, but detectable, levels of T-bet and GATA-3 transcripts, and all other samples. nd, not detected (>40 cycles without amplification). Numbers indicate fold differences between individual samples and 0 h *IFN-γR*$^{-/-}$ B cells. *D*, Purified naive B cells isolated from WT mice or STAT1-deficient mice were cultured with OVA-specific Th1 cells and Ag (anti-IgM + OVA peptide). B cells were repurified at the indicated times, and mRNA was isolated. T-bet mRNA levels were determined by qPCR. The data are presented as the fold difference between naive (0 h) *STAT1*$^{-/-}$ B cells and all other samples. T-bet was detected in both *STAT1*$^{-/-}$ and *STAT1*$^{+/+}$ B cells. Data are representative of three independent experiments.

we observed a 2-fold decrease in T-bet mRNA levels in *STAT1*$^{-/-}$ B cells compared with WT B cells, T-bet transcripts were induced >40-fold in *STAT1*$^{-/-}$ B cells, but only 20-fold in WT B cells. Thus, T-bet expression in B cells appears to be controlled by an IFN-γR-dependent, but STAT1-independent, mechanism.

IFN-γ production by Be1 cells is regulated by a T-bet-dependent mechanism

T-bet expression is obligate for IFN-γ production by CD4 T cells activated with Ag in the presence of IFN-γ or IL-12 (12). Because we found that T-bet expression in B cells is regulated by IFN-γR-dependent signals, we hypothesized that T-bet would be required for IFN-γ production by Be1 cells. To test this hypothesis, we purified naive B cells isolated from WT or *T-bet*$^{-/-}$ mice and cultured the cells with OVA-specific Th1 effector cells, anti-IgM, and OVA peptide. After 4 days, we purified the B cells, restimulated the cells, and measured IFN-γ production. A significant fraction (14%) of WT Be1 cells was able to produce IFN-γ upon restimulation, whereas <1% of T-bet-deficient B cells, cultured under the same conditions, were able to produce IFN-γ (Fig. 4*A*). In agreement with these findings, we detected <1 ng/ml secreted IFN-γ in T-bet-deficient B cell cultures, whereas WT B cells produced >100 ng/ml IFN-γ (Fig. 4*B*). Importantly, the T-bet-deficient B cells expanded normally (data not shown) and produced TNF-α and elevated levels of IL-2 upon restimulation (Fig. 4), indicating that T-bet is not necessary for the survival or expansion of cytokine-producing B cells. However, T-bet is required for the development of IFN-γ-producing Be1 cells.

T cell and B cell-derived IFN-γ are both necessary for the full differentiation of IFN-γ-secreting Be1 cells

Because the development of IFN-γ-producing Be1 cells is dependent on the expression of the IFN-γR by B cells, IFN-γ must be required for the Th1-induced differentiation of naive B cells into Be1 cells. To determine whether T cell-derived IFN-γ is required for the differentiation of Be1 cells, we tested whether naive B cells would differentiate into IFN-γ-producing Be1 cells in cultures containing IFN-γ-deficient Th1 cells and Ag. To accomplish this, we made IFN-γ-deficient Th1 effectors by culturing naive T cells from *IFN-γ*$^{-/-}$ PCCF-specific TCR Tg mice with anti-CD3 and anti-CD28 under Th1-polarizing conditions. As expected, the IFN-γ-deficient T cells activated and expanded normally in the presence of the exogenously added cytokines, but were unable to secrete IFN-γ upon restimulation (data not shown). Importantly, however, the IFN-γ-deficient Th1 effector cells appeared phenotypically identical to the normal Th1 effectors and were able to produce equivalent levels of cytokines other than IFN-γ (data not shown). We then cultured IFN-γ-deficient or WT Th1 effector cells with purified naive B cells from either WT B10.BR mice or IFN-γ-deficient B10.BR mice and anti-IgM and PCCF peptide. After 4

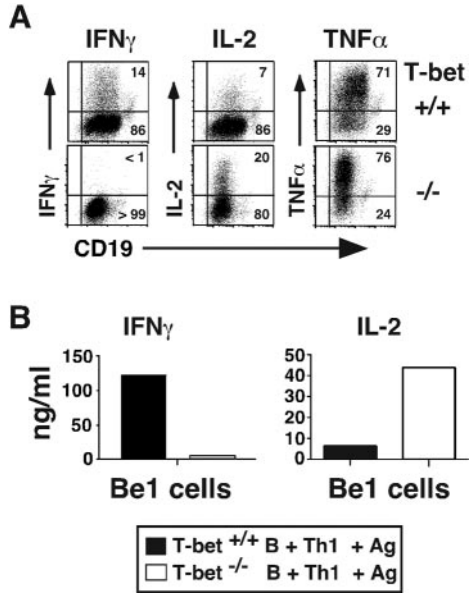

FIGURE 4. Production of IFN-γ by Be1 cells is T-bet dependent. *A* and *B*, Purified naive B cells from C57BL/6J mice or T-bet-deficient mice were cultured with OVA-specific Th1 cells and Ag (anti-IgM + OVA peptide) for 96 h. B cells were purified (>99% $CD19^+$) and restimulated for 10 h, and cytokine production (IFN-γ, IL-2, and TNF-α) by $CD19^+$ Be1 cells was analyzed after 8 h by ICCS (*A*) or after 72 h by ELISA (*B*). Data are representative of two independent experiments.

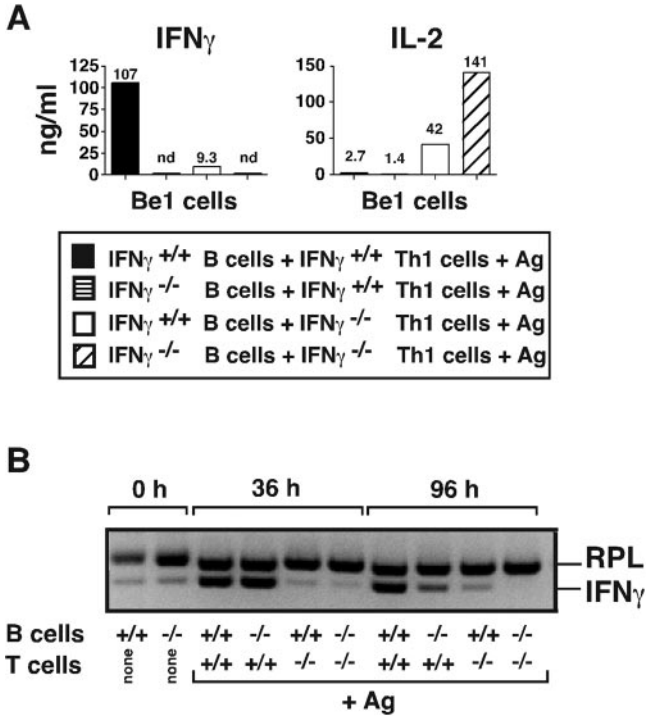

FIGURE 5. T cell- and B cell-derived IFN-γ are both required for the development and maintenance of IFN-γ-producing Be1 cells. *A*, Purified naive B cells from B10.BR mice or from IFN-γ-deficient B10.BR mice were cultured with PCCF-specific Th1 effectors or IFN-γ-deficient PCCF-specific Th1 effector cells in the presence of HEL plus PCCF. At 96 h, B cells were purified (>99% $CD19^+$) and restimulated for 10 h, and cytokine levels were determined. *B*, Naive B cells from WT (+/+) or IFN-γ-deficient (−/−) mice were cultured with PCCF-specific TCR Tg Th1 effector cells (+/+) or IFN-γ-deficient PCCF-specific Th1 effectors (−/−) in the presence of HEL plus PCCF. cDNA was prepared from purified B cells (>99% $CD19^+$) at 0, 36, and 96 h, and semiquantitative PCR was performed using IFN-γ-specific primers that amplify the truncated IFN-γ transcripts produced by *IFN-*$\gamma^{-/-}$ cells. The housekeeping gene RPL32 was amplified in the same PCR as a control, and PCR products were resolved on a 2% agarose gel.

days, we purified the B cells, restimulated the cells, and measured IFN-γ production by ELISA. As expected IFN-γ-deficient B cells cultured with either WT or IFN-γ-deficient Th1 cells did not secrete detectable levels of IFN-γ upon restimulation, although WT B cells cultured with WT Th1 cells produced >100 ng/ml IFN-γ (Fig. 5*A*). In contrast, WT B cells cultured with IFN-γ-deficient Th1 cells produced ~10-fold less IFN-γ (9.3 ng/ml) upon restimulation (Fig. 5*A*), indicating that IFN-γ derived from Th1 cells is required for the generation of IFN-γ-producing Be1 cells. Interestingly, and in agreement with our previous data using IFN-γR-deficient and T-bet-deficient B cells (Figs. 2*A* and 4*B*), WT and IFN-γ-deficient B cells produced significantly more IL-2 when cultured with IFN-γ-deficient Th1 cells (Fig. 5*A*).

Although IFN-γ derived from Th1 cells appeared to play a critical role in promoting the generation of IFN-γ-producing Be1 cells, B cells up-regulate IFN-γ mRNA in <24 h (Fig. 1*A*). Thus, the IFN-γ derived from the B cell itself could potentially play a role in sustaining its own expression via an IFN-γR autocrine feedback loop. To test this hypothesis, WT or IFN-γ-deficient Th1 effector cells were cultured with purified naive WT or IFN-γ-deficient B cells in the presence of Ag. At 36 and 96 h, the cultures were harvested, and mRNA was isolated from repurified effector B cells. To analyze the expression of the IFN-γ gene in IFN-γ-deficient B cells, we developed a semiquantitative PCR assay that amplified the 5′-proximal region of the IFN-γ transcript using a forward primer within the 5′-untranslated region of the mRNA and a reverse primer that spanned exons 1 and 2 of the IFN-γ gene. This approach allowed us to specifically amplify IFN-γ mRNA (and not genomic DNA) in a region upstream of the targeted deletion of the IFN-γ gene, which is located in the distal end of exon 2 (38). Importantly, we could detect the PCR product in naive WT and IFN-γ-deficient B cells (Fig. 5*B*), indicating that this region of the gene is transcribed in both WT and IFN-γ-deficient cells. After culturing the cells for 36 h, IFN-γ mRNA was strongly up-regulated in WT as well as IFN-γ-deficient B cells, provided the cultures contained WT IFN-γ-producing Th1 cells (Fig. 5*B*). However, we did not observe significant up-regulation of IFN-γ mRNA at 36 h in either WT or IFN-γ-deficient B cells when the B cells were cocultured with IFN-γ-deficient Th1 cells (Fig. 5*B*), indicating that T cell-derived IFN-γ is required for the initial development of IFN-γ-expressing Be1 cells. At 96 h, WT B cells cocultured with WT Th1 cells continued to maintain elevated IFN-γ transcript levels (Fig. 5*B*). In striking contrast, IFN-γ mRNA levels were not sustained at 96 h in IFN-γ-deficient B cells even when cultured with WT Th1 cells (Fig. 5*B*). Furthermore, the IFN-γ transcripts were maintained at the lowest levels when both T and B cells were unable to produce IFN-γ (Fig. 5*B*). Together, these data indicate that the IFN-γ produced by T cells is not sufficient to sustain Be1 differentiation, and autocrine production of IFN-γ is required to maintain high level transcription of the IFN-γ gene during effector Be1 cell differentiation.

IFN-γ-producing Be1 cells develop normally in the absence of IL-12 and STAT-4

T-bet is a master regulator of Th1 development and not only controls IFN-γ/IFN-γR-induced Th1 development, but also up-regulates expression of the IL-12Rβ2 chain and allows T cells to become responsive to IL-12 (24, 30). Because B cells can also

produce IL-12 (3, 39–41), we tested whether production of IL-12 by the differentiating Be1 cells is required for IFN-γ production by Be1 cells. Therefore, we added anti-IL-12-blocking Ab to the cultures containing naive B cells, OVA-specific Th1 cells, and Ag. On day 4, we purified and restimulated the Be1 effectors and measured IFN-γ production. Addition of anti-IL-12 Ab had no effect on the amount of IFN-γ produced by Be1 cells (Fig. 6*A*) or on the frequency of IFN-γ-producing Be1 cells as determined by ICCS (Fig. 6*B*). Similarly, we found that STAT4-deficient B cells (on the BALB/c, low IFN-γ-producing genetic background) produced as much IFN-γ as WT BALB/c Be1 cells when first primed by BALB/c Th1 cells and Ag (Fig. 6*C*). Together, these data indicate that the development of IFN-γ-producing Be1 cells is not dependent on autocrine production of IL-12 or signaling through the IL-12R/STAT4 pathway.

IL-12-induced production of IFN-γ by B cells is T-bet dependent and requires expression of the IFN-γR

Although Th1 cell-induced Be1 development is not dependent on IL-12 or STAT4, B cells activated with Ag or TLR ligands in the presence of IL-12 or IL-12 plus IL-18 produce IFN-γ (3, 9, 11, 39). To test whether IFN-γ production by B cells activated in the presence of IL-12 is also T-bet dependent, we cultured T-bet-deficient B cells and WT B cells in the presence of IL-12 with a number of different activators including anti-IgM, anti-CD40, BAFF, IFN-γ, plus anti-IL-4 (Fig. 6*D*) as well as IL-18, IL-18 plus

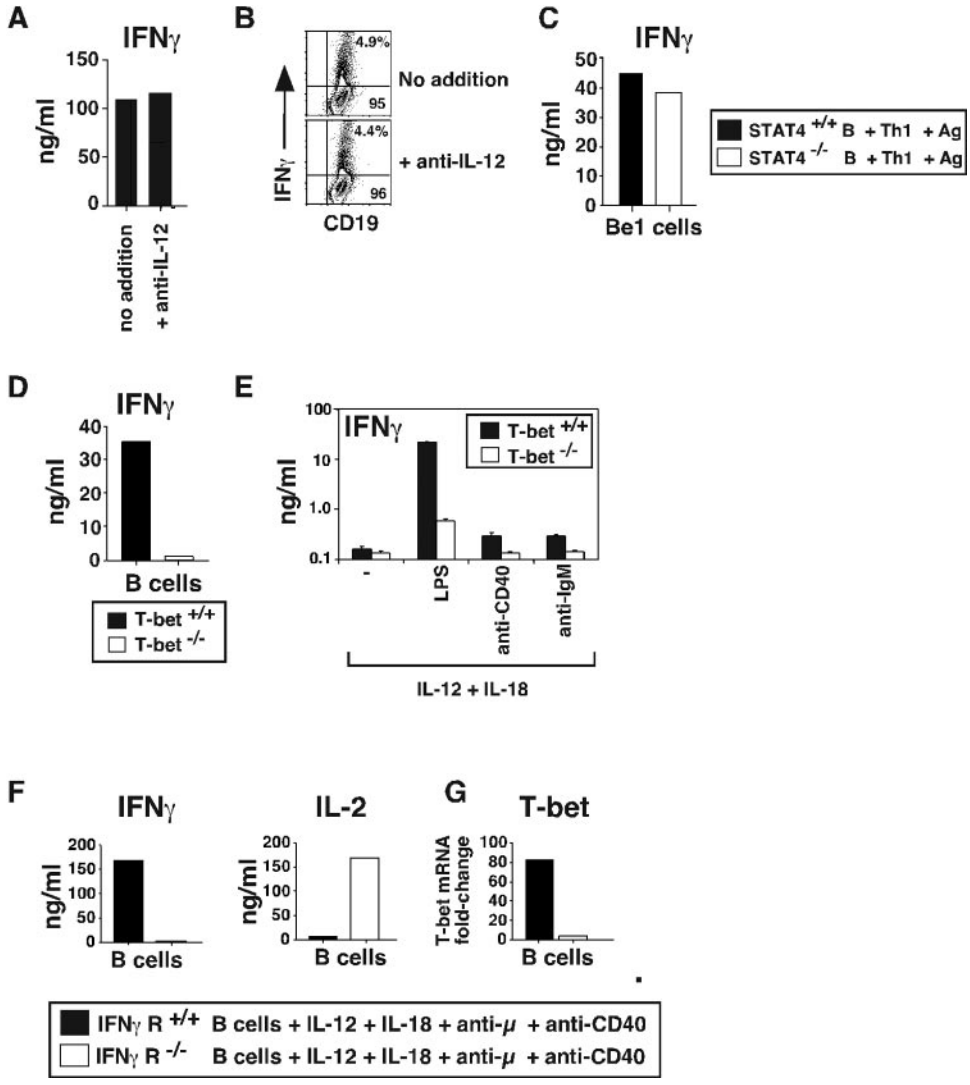

FIGURE 6. IL-12-induced IFN-γ production by B cells is dependent on IFN-γR and T-bet. *A*, Purified naive B cells isolated from C57BL/6J mice were cultured with OVA-specific Th1 cells and Ag (anti-IgM + OVA peptide) in the presence or the absence of anti-IL-12 Ab for 96 h. B cells were purified and restimulated for 10 h, and IFN-γ levels in the supernatant (*A*) or in the $CD19^+$ cells (*B*) were determined. *C*, Purified naive B cells isolated from BALB/c mice or STAT4-deficient mice were cultured with HA-specific Th1 effectors and Ag (anti-IgM + HA peptide) for 96 h. The B cells were purified (>99% $CD19^+$) and restimulated for 10 h, and IFN-γ levels were determined. *D*, Purified naive B cells from C57BL/6J mice or T-bet-deficient mice were cultured with anti-CD40 and anti-IgM in the presence of IL-12, IFN-γ, BAFF, and anti-IL-4. After 96 h, B cells (>99% $CD19^+$) were washed and restimulated for 10 h, and IFN-γ levels were determined. *E*, Purified naive B cells from C57BL/6J mice or T-bet-deficient mice were cultured with LPS, anti-CD40, or anti-IgM in the presence of IL-12 and IL-18. Supernatants were removed at 72 h, and IFN-γ levels were determined. *F*, Purified naive B cells from C57BL/6J mice or IFN-γR-deficient mice were cultured with anti-IgM and anti-CD40 in the presence of IL-12 and IL-18. After 96 h, B cells were purified and restimulated for 10 h, and cytokine levels in the supernatant were determined. *G*, Purified naive B cells from C57BL/6J mice or IFN-γR-deficient mice were cultured with anti-IgM and anti-CD40 in the presence of IL-12 and IL-18. After 48 h, B cells were repurified, and mRNA was isolated. Tbet mRNA levels were determined by qPCR. The data are presented as the fold difference between *IFN-γR*$^{-/-}$ B cells and *IFN-γR*$^{+/+}$ B cells. T-bet was detected in IFN-γR$^{-/-}$ B cells. Data are representative of two or more independent experiments.

anti-CD40, IL-18 plus LPS, and IL-18 plus anti-IgM (Fig. 6*E*) and then measured IFN-γ after the cells were restimulated with PMA plus Ca^{2+} ionophore. As shown in Fig. 6*D*, WT B cells stimulated with several B cell activators, including anti-CD40, BAFF, and anti-IgM, produced easily measurable levels of IFN-γ upon restimulation (~35 ng/ml); however, the T-bet-deficient B cells made only minimal amounts of IFN-γ (~1 ng/ml). Likewise, we found that IFN-γ production by T-bet-deficient B cells was always <1 ng/ml even when the B cells were stimulated with IL-12 plus IL-18 in the presence of LPS, anti-CD40, or anti-IgM (Fig. 6*E*). Thus, even when B cells are stimulated by an array of different activators, IL-12-mediated IFN-γ production is highly dependent on T-bet.

It is not known whether the IFN-γR signaling pathway is used during IL-12-induced IFN-γ production by B cells. To address this question, we stimulated purified naive B cells from WT and *IFN-γR*$^{-/-}$ mice with anti-IgM and anti-CD40 in the presence of IL-12 and IL-18 for 4 days and then measured IFN-γ production in response to restimulation. WT B cells activated with IL-12 and IL-18 secreted substantial amounts of IFN-γ and expressed high levels of T-bet, whereas IFN-γR-deficient B cells stimulated in an identical manner produced significantly lower amounts of IFN-γ (<10 ng/ml) and expressed very low levels of T-bet mRNA (Fig. 6, *F* and *G*). As we observed with Th1 cell-induced activation, IL-12-induced activation of IFN-γR-deficient B cells resulted in the production of large amounts of IL-2 (Fig. 6*F*) and higher cell recoveries (data not shown). These data indicate that both Th1 cell-driven and IL-12-mediated IFN-γ production by B cells require signaling through the IFN-γR and T-bet. Therefore, these molecules act as master regulators of IFN-γ gene transcription in B cells.

Discussion

Although B cells produce immunoregulatory cytokines such as IFN-γ in the context of infection and autoimmune disease, very little is known about the mechanisms that control cytokine production by B cells. In this study we have identified multiple factors that facilitate IFN-γ production by B cells. Interestingly, just as has been previously observed for CD4 T cells (42), Ag receptor signals and genetically determined factors modulate the levels of IFN-γ produced by Be1 cells, but do not control Be1 differentiation. Instead, we found that a cognate interaction between T and B cells was necessary for Be1 development and survival, indicating that the Th1 effector cells must provide costimulatory signals and/or cytokines to the developing Be1 cells. In support of this hypothesis, we found that IFN-γ produced by Th1 effector cells was required for the development of Be1 cells, because we observed a 95% reduction in the amount of IFN-γ produced by restimulated Be1 cells that were initially primed by IFN-γ-deficient Th1 cells. Although T cell-derived IFN-γ is clearly required for the differentiation of IFN-γ-producing B cells, it is not sufficient for full Be1 development, because B cells activated in culture with Ag and exogenous IFN-γ do not differentiate into high rate IFN-γ-producing Be1 cells (D. P. Harris and F. E. Lund, unpublished observations). Although we have not yet analyzed the role of T cell-derived accessory/costimulatory molecules in detail, we know that interactions between CD154 on Th1 cells and CD40 on B cells is not required for Be1 differentiation, because inclusion of the CD154-blocking Ab MR1 in our cultures had no impact of the development of Be1 cells in our model system (2). Finally, even though IFN-γ production by Th1 cells is required for Be1 development, it is not required for the generation of effector B cells capable of expanding and producing cytokines, because B cells primed in the absence of IFN-γ-derived signals actually survived/expanded better than B cells cultured with IFN-γ-producing Th1 cells, and these B cell produced very large quantities of IL-2.

Our data clearly show that IFN-γ production by Be1 cells is positively regulated by an IFN-γ/IFN-γR-dependent pathway, although IL-2 production is negatively regulated by this same pathway. The IFN-γ/IFN-γR pathway is well described for CD4 cells and has been shown to be controlled by the transcription factors T-bet and STAT1 (24, 29). Similar to what has been shown for CD4 cells, we found that the development of IFN-γ-producing Be1 cells is critically dependent on T-bet, at least on the C57BL/6 genetic background. Interestingly, we also showed that IL-2 production by Be1 cells is negatively regulated by T-bet. It has been previously demonstrated that T-bet expression can repress IL-2 transcription, particularly in Th2 cells (26), and this may explain our previous observations that Be2 cells, which express very low levels of T-bet and produce only small quantities of IFN-γ, consistently produce 5- to 10-fold more IL-2 than Be1 cells (3).

Our data show that signals through the IFN-γR are required for T-bet up-regulation in B cells that are activated with Th1 cells and Ag. This is in apparent contrast to B cells that have been activated with CpG oligonucleotides (39) or IL-27 (43), both of which can up-regulate T-bet via an IFN-γ-independent mechanism. Thus, there are probably multiple mechanisms to enhance T-bet expression by B cells, including an IL-27/STAT1 coupled pathway (43), a TLR9/MyD88/NF-κB pathway (39), and the IFN-γR-dependent pathway described in this study. However, regardless of how the B cells were initially activated, we predict that once T-bet has been activated, IFN-γ production by the responding B cells will be sustained by a T-bet/IFN-γR-dependent mechanism.

Although T-bet regulates IFN-γ production in both CD4 Th1 cells and Be1 cells activated in a cognate fashion with Ag, T-bet expression appears to be controlled by a novel mechanism in B cells. For example, although T-bet expression is regulated by the IFN-γR in both CD4 T cells and B cells, in Th1 cells, T-bet expression is regulated by an IFN-γR/STAT1-dependent signaling pathway (12, 25). However, in B cells activated with Ag and Th1 cells, T-bet expression is not critically dependent on STAT1 coupled to the IFN-γR. One caveat to these experiments is that the STAT1-deficient B cells were isolated from 129S6/SvEv mice, rather than from C57BL/6J or B10.BR animals. However, 129/SvEv B cells, like B10.BR and C57BL/6J B cells, are high IFN-γ producers (compare Figs. 1*B* and 2*B*), and although T-bet expression was 2-fold lower in activated STAT1-deficient 129/SvEV B cells compared with WT 129/SvEV B cells, significant up-regulation (40-fold increase) of T-bet was observed within 24 h of activation of STAT1-deficient B cells. Thus, these data strongly suggest that IFN-γ production can be regulated by biochemically related, but distinct, mechanisms in B cells compared with CD4 T cells.

Under normal circumstances, it is likely that STAT1 mediates signaling through the IFN-γR in B cells; however, our data show that in the absence of STAT1, B cells activated with Ag and cognate Th1 cell help are still able to signal through the IFN-γR to up-regulate T-bet. Although we do not know how T-bet is up-regulated in STAT1-deficient Be1 cells, it is possible that in B cells, other STAT molecules (or perhaps novel factors) are able to mediate IFN-γR signaling. In support of this hypothesis, STAT3 is activated by IFN-γ in the absence of STAT1 in T cells (44), and a number of studies using T cells have demonstrated STAT 1-independent induction of IFN-γ (24, 45–47) as well as several IFN-γ-inducible genes (44). Furthermore, it has been shown that the absence of STAT1 leads to either normal or enhanced IFN-γ production by T cells and NK cells during infections with *Toxoplasma gondii* or lymphocytic choriomeningitis virus (46, 47). Likewise,

we also found that STAT1-deficient B cells often produced as much or even more IFN-γ than their WT counterparts. Alternatively, it is possible that the very low levels of T-bet expressed by STAT1-deficient B cells (39) (Fig. 3*D*) are sufficient to promote the initial production of IFN-γ and to initiate the autocrine feedback loop through the IFN-γR. In fact, we have observed that several non-CpG TLR ligands induce minimal expression of T-bet and yet are still able to induce a T-bet-dependent IgG2a response (S. L. Peng, unpublished observation). In further support of this hypothesis, our data clearly show that sustained transcription of the IFN-γ gene by B cells is dependent on an autocrine feedback loop. Thus, although Th1 cells are needed to initiate IFN-γ production by developing Be1 cells, IFN-γ produced by B cells is needed to sustain high rate transcription of the IFN-γ gene, at least in culture. Although it has been previously proposed by many groups that an IFN-γ-driven positive feedback loop must regulate IFN-γ production by Th1 cells (12), our data are the first to show that this autocrine feedback loop directly regulates IFN-γ at the transcriptional level.

Interestingly, we found that IFN-γ production by B cells activated in the presence of IL-12 is also controlled by an IFN-γR/T-bet-dependent feedback loop. In fact, IFN-γ production by IL-12-stimulated B cells is T-bet dependent regardless of whether the B cells are coactivated with IL-18, LPS, anti-CD40, or T cells and Ag. This result is similar to that seen with IL-12-stimulated T-bet-deficient T cells, where it was shown that T-bet activation occurs upstream of the IL-12R/STAT4 pathway (30). Likewise, it is compatible with data showing that IL-12-mediated up-regulation of T-bet in human B cells is also dependent on IFN-γ (48). Our data extend these findings to show that T-bet up-regulation as well as IFN-γ production by B cells activated in the presence of IL-12 and IL-18 are absolutely dependent on the expression of IFN-γR. Thus, the data suggest that IL-12R signaling leads to initial activation of the IFN-γ gene, which, in turn, leads to IFN-γR signaling, T-bet up-regulation, and full transcriptional activation of the IFN-γ gene locus. In agreement with this hypothesis, Durali et al. (48) have shown that treatment of IL-12-activated human B cells with anti-IFN-γ Abs blocks the induction of T-bet.

Based on all these results, we propose a working model to describe the molecular mechanisms that regulate IFN-γ production by B cells (Fig. 7). Naive B cells express very low levels of the transcriptional regulators T-bet and GATA-3 and do not express Eomes (D. P. Harris, S. Goodrich, and F. E. Lund, unpublished observations), another T-box factor reported to play an important role in Tc1 development (49). When B cells present Ag to Th1 cells, the activated Th1 cells secrete IFN-γ, which subsequently binds to the IFN-γR on B cells and induces a STAT1-independent signal that results in the up-regulation of T-bet and the down-regulation of GATA-3. T-bet then induces transcriptional activation of the IFN-γ gene and IFN-γ production by B cells. IFN-γ produced by the B cell in response to T cell-derived IFN-γ leads to the initiation of a positive feedback loop and sustained production of IFN-γ by Be1 cells. Similarly, B cells responding to T-independent stimuli in the presence of IL-12 and IL-18 produce IFN-γ, again through an IFN-γR/T-bet-dependent positive feedback loop. Thus, both T cell-dependent and T cell-independent IFN-γ production by B cells are mediated by feedback loops that are ultimately controlled by the IFN-γR and T-bet, but are not dependent on STAT1.

Together, these data suggest that the development of Be1 cells, like that of Th1 cells, is regulated in a complex fashion, with many different factors contributing to the differentiation and expansion of effector B cells that are competent to produce large quantities of IFN-γ. Given that Ag-specific Th1 cells can provide all the signals necessary for full differentiation of IFN-γ-producing Be1 cells and that Be1 effectors can provide all the signals necessary for Th1 differentiation from naive T cells (3), it is likely that the cognate interactions that occur between cytokine-producing B and T cells can result in an amplification of type 1 immune responses. In the case of immune responses to bacterial or viral pathogens, this is likely to be beneficial; however, in the case of immune responses to autoantigens, it may result in amplification of the autoimmune loop and increased pathology.

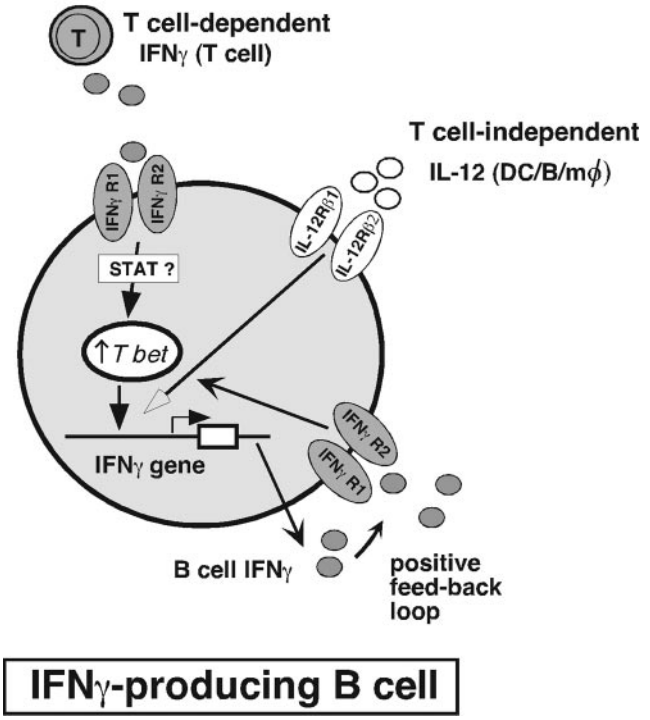

FIGURE 7. IFN-γ and T-bet are master regulators of IFN-γ production by B cells. Naive B cells express low levels of T-bet, GATA-3, and IFN-γ as well as IFN-γR and IL-12R. Naive B cells can be activated by a T cell-dependent pathway (solid bold arrows) or by a T cell-independent pathway (open arrows) to produce IFN-γ. Upon encounter with IFN-γ-producing Th1 cells, B cells up-regulate T-bet in response to an IFN-γR-dependent signal. This signal is presumably dependent on a STAT or STAT-like molecule, but is not critically dependent on STAT1. Up-regulation of T-bet by IFN-γR-driven signals subsequently increases IFN-γ gene transcription by B cells. B cell-derived IFN-γ initiates a positive feedback loop controlled by IFN-γR and T-bet. B cells, activated by T-independent stimuli in the presence of IL-12, receive an IL-12R-dependent signal, which induces IFN-γ gene transcription by the B cells. B cell-derived IFN-γ initiates a positive feedback loop controlled by IFN-γR and T-bet.

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Disclosures

The authors have no financial conflict of interest.

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