This information is current as of April 14, 2017.

Type I IFN Induces Binding of STAT1 to Bcl6: Divergent Roles of STAT Family Transcription Factors in the T Follicular Helper Cell Genetic Program

Shingo Nakayamada, Amanda C. Poholek, Kristina T. Lu, Hayato Takahashi, Masanari Kato, Shigeru Iwata, Kiyoshi Hirahara, Jennifer L. Cannons, Pamela L. Schwartzberg, Golnaz Vahedi, Hong-wei Sun, Yuka Kanno and John J. O'Shea

*J Immunol* 2014; 192:2156-2166; Prepublished online 31 January 2014;
doi: 10.4049/jimmunol.1300675
http://www.jimmunol.org/content/192/5/2156

**References**

This article cites 90 articles, 42 of which you can access for free at:
http://www.jimmunol.org/content/192/5/2156.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Type I IFN Induces Binding of STAT1 to Bcl6: Divergent Roles of STAT Family Transcription Factors in the T Follicular Helper Cell Genetic Program

Shingo Nakayamada,*1,2 Amanda C. Poholek,*1 Kristina T. Lu,† Hayato Takahashi,*3 Masanari Kato,* Shigeru Iwata,* Kiyoshi Hirahara,*4 Jennifer L. Cannons,† Pamela L. Schwartzberg,† Golnaz Vahedi,* Hong-wei Sun,‡ Yuka Kanno,* and John J. O'Shea*  

CD4+ T follicular helper cells (TFH) are critical for the formation and function of B cell responses to infection or immunization, but also play an important role in autoimmune. The factors that contribute to the differentiation of this helper cell subset are incompletely understood, although several cytokines including IL-6, IL-21, and IL-12 can promote TFH cell formation. Yet, none of these factors, nor their downstream cognate STATs, have emerged as nonredundant, essential drivers of TFH cells. This suggests a model in which multiple factors can contribute to the phenotypic characteristics of TFH cells. Because type I IFNs are often induced by IFNs (IFN-α/β) promote Th1 responses, thus one possibility was these factors antagonized TFH-expressed genes. However, we show that type I IFNs (IFN-α/β) induced B cell lymphoma 6 (Bcl6) expression, the master regulator transcription factor for TFH cells, and CXCR5 and programmed cell death-1 (encoded by Pdcd1), key surface molecules expressed by TFH cells. In contrast, type I IFNs failed to induce IL-21, the signature cytokine for TFH cells. The induction of Bcl6 was regulated directly by STAT1, which bound to the Pdcd1 promoter. The expression of Bcl6 is essential for TFH cell differentiation, and its absence leads to severe defects in TFH cell formation and function. These findings provide insights into the regulation of TFH cell development and function, and highlight the importance of type I IFNs in the induction of TFH cells.

Received for publication March 13, 2013. Accepted for publication January 3, 2014.

The master regulator transcription factor required for TFH cell formation is the transcriptional repressor B cell lymphoma 6 (Bcl6) (28–30). In the absence of Bcl6, TFH cells are unable to form, and subsequently GCs are not present (28). Like other master regulators, overexpression of Bcl6 not only enforces TFH cell differentiation but also attenuates differentiation to other fates by repressing the expression of master transcription factors for other CD4+ T cell subsets, including T-bet, GATA3, and Rorγt (29, 31).

CD4+ T cell subset differentiation is mediated in large part by exposure to various cytokines. For example, Th1 cells develop in the presence of IL-12 and IFN-γ, whereas Th2 cells form after exposure to IL-4 (1). For TFH cells, several cytokines have been reported to effect differentiation. In vitro, exposure of CD4+ T cells to the cytokines IL-6 and IL-21 drives a TFH-like phenotype (30, 31).
32–35), but in vivo, deletion or neutralization of IL-6 and IL-21 does not completely ablate TFH cell generation and GC formation (36–41). This suggests that although these factors may be sufficient to achieve differentiation, they are not necessary, implying that other cytokines can contribute to TFH cell differentiation. Many cytokines that drive helper T cell specification bind type I/II cytokine receptors, which activate JAKs and STAT family transcription factors to translate the cytokine signal into specific programs of gene transcription that mediate effector differentiation (42, 43). Both IL-6 and IL-21 act through STAT3 (44, 45) and in circumstances where STAT3 signaling is impaired, TFH cell formation is reduced (34, 46). Although in vivo STAT3 is not absolutely critical for TFH cell formation, STAT3 clearly plays a positive role in promoting the TFH cell program. These data add to the argument that signals other than IL-6, IL-21, and STAT3 can contribute to TFH cell induction. Human CD4+ T cells exposed to IL-12 acquire an increased capacity to help B cells in vitro and express many TFH signature genes (47). Studies have differed looking at patients with IL-12Rβ1 mutations. Although one study reported reduced circulating memory T cell numbers, another found normal numbers (46, 48). Regardless, naive T cells from these patients are impaired in their ability to develop into functional TFH cells after exposure to IL-12 (46). In addition, murine CD4+ T cells cultured with IL-12 acquire TFH cell characteristics early in a STAT4-dependent manner; yet consistent exposure to IL-12 increases expression of T-bet and promotes Th1 cell differentiation (49). Like STAT3 or IL-6 and IL-21 deficiency, the absence of IL-12 and STAT4 in murine models has only a modest effect on TFH cell development in vivo (49). These data further support the contention that there is redundancy in the cytokines and STATs that control TFH cell formation and further argue for the role of additional factors in TFH cell differentiation. Although it appears that multiple cytokines can promote TFH differentiation, IL-2 interferes with TFH cell formation (50–53). Two nonmutually exclusive mechanisms have been proposed: in the first, IL-2 acting via STAT5 induces the transcriptional repressor Blimp-1, which serves to repress Bcl6 and TFH cell formation (51, 52). In addition, active STAT5 can displace STAT3 and disrupt the balance of signals a CD4+ T cell receives during differentiation plays a critical role in driving this effector program. Type I IFNs (IFN-α/β) are ubiquitous cytokines produced by innate immune cells early in infection (54). These critical antiviral cytokines also have key immunoregulatory roles. During chronic viral infection, IFNs have paradoxical roles, both promoting control of viral replication and mediating immunosuppressive pathways that limit viral control (55–57). In T cells, IFN-α/β activates STAT1 and to a lesser extent STAT4, inducing T-bet and IFN-γ (58–60). In vivo, IFN-α/β contributes to T cell survival and clonal expansion during viral infection, and it can support Th1 differentiation by synergizing with IL-12 and IFN-γ (61, 62). Yet, when IFN-α/β is compared with IL-12, the classic cytokine for Th1 differentiation, it is not sufficient to induce full Th1 cell development (63). In addition to its effects on Th1 cells, an indirect role for type I IFN in TFH cell differentiation has been demonstrated in vivo. Reduced IL-6 levels in the absence of type I IFN signaling in dendritic cells resulted in impaired TFH cell formation (64). In addition, IFN signaling through dendritic cells promotes both primary and long-lived Ab responses (65). These findings suggested the importance of type I IFNs in humoral immune responses. However, the direct effects of type I IFNs in TFH differentiation have not been carefully explored; given its ability to induce T-bet, it might be anticipated that it would antagonize expression of TFH-associated genes.

In this study, we set out to examine the role of type I IFNs on TFH cell differentiation. We found that type I IFNs induced Bcl6 and promoted CXCR5 and PD-1 expression, but unlike IL-6 and IL-12, IFN-α/β did not induce IL-21 secretion. In conjunction with IL-6, however, IFN-α/β enhanced IL-21 production. The ability of IFN-α/β to drive these TFH cell features was entirely STAT1-dependent and STAT1 bound directly to the Bcl6 locus. This suggests type I IFNs and STAT1 promote some, but not all aspects of TFH cell development. Taken together, these findings help explain some of the apparent redundancy in the requirements for factors that induce TFH cell responses.

Materials and Methods

Mice, cell isolation, and cell culture

C57BL/6d were purchased from The Jackson Laboratory (Bar Harbor, ME). Stat1−/− mice were provided by D.E. Levy (New York University, New York, NY) (67). All mice were handled in accordance with the guidelines of the National Institute of Allergy and Infectious Diseases and the National Institutes of Health. All experiments were approved by the National Institutes of Health Animal Care and Use Committee. Splenic and lymph node T cells were obtained by disrupting organs of 8- to 12-wk-old mice. All cell cultures were performed in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, and 2.5 μM ME-2–ME. T cells were enriched with a CD4+ T cell kit and AutoMacs isolator (Miltenyi Biotec). Naive CD4+ T cells were stained with Abs to CD4, CD62L, CD44, and CD25 (BD Biosciences and eBioscience), and isolated by flow cytometry cell sorting using a BD Biosciences FACSAria IIIu. Isolated cells were cultured with plate-bound anti-CD3 and anti-CD28 (10 μg/ml each) under neutral (no cytokines), IL-6 (20 ng/ml), IL-12 (10 ng/ml), IFN-α (5000 U/ml), or IFN-β (5000 U/ml) conditions for 3 d and then cultured further in IL-2 (50 U/ml) alone (neutral condition) or in combination with IL-6, IL-12, IL-16, IFN-α, or IFN-β and grown an additional 2 d. For T-bet coculture, naive B cells were sorted and cultured with T cells that had been cultured for 5 d as above at a 1:3 T:B ratio without stimulation or on plates coated with anti-CD3 and anti-CD28 (stimulated) for 4 d. Cytokines were from R&D Systems. Anti-CD3 and anti-CD28 were from eBioscience.

Intracellular staining and flow cytometry

For intracellular cytokine staining, cells were restimulated for 2 h with 50 ng/ml PMA and 1 μg/ml ionomycin with the addition of brefeldin A (GolgiStop; BD Biosciences), then fixed and permeabilized with Cytofix/CytoPerm solution (BD Biosciences). Intracellular IL-21 staining was performed using soluble IL-21R/Fc (R&D Systems), followed by PE-labeled affinity-purified F(ab’)2 fragment of goat anti-human Fcy (Jackson ImmunoResearch Laboratories) (68), then further stained with PerCP-Cy5.5 anti-CD4 and APC anti–IFN-γ (BD Biosciences). The following Abs were used for cell surface or intracellular staining; PE anti–PD-1, PE anti–ICOS, PerCP-Cy5.5 anti–CD4, PE anti–Bcl6, and Alexa Fluor-647 anti–T-bet (eBioscience). CXCR5 staining was done using biotinylated anti–CXCR5 for 1 h, followed by APC-labeled streptavidin (BD Biosciences). For phosphorylated STAT staining, freshly isolated naive T cells were incubated for 30 min alone (no stimulation) or with IL-6 (20 ng/ml), IFN-α (5000 U/ml), or IFN-β (5000 U/ml). Cells were fixed in FoxFlx Buffer (BD Biosciences) and permeabilized in Perm Buffer III (BD Biosciences), then stained with the following Abs, FITC anti–p-STAT1, PE anti–p-STAT3, APC anti–p-STAT4 (BD Biosciences). Stained cells were analyzed on a flow cytometer (FACSCanto; BD Biosciences). Events were collected and analyzed with FlowJo software (Tree Star).

Quantitative real-time PCR

Total RNA was isolated with the use of the mirVana miRNA kit (Applied Biosystems/Ambion); CDNA was synthesized with the TaqMan Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed with an ABI PRISM 7500 sequence detection system with site-specific primers and probes (Applied Biosystems). The comparative threshold cycle method and an internal control (β-actin) were used to normalize the expression of the target genes. List of primers and probes from Applied Biosystems: mouse ACTB, 4352541E; Bcl6, Mm01342164_m1; Tbx21,
Chromatin immunoprecipitation-sequencing and chromatin immunoprecipitation-quantitative PCR

Chromatin immunoprecipitation (ChIP)–sequencing (seq) experiments and data processing were performed as previously described (69, 70) using anti-STAT1 Ab (sc-592; Santa Cruz Biotechnology). Briefly, ~10 ng DNA was recovered from 10 million cells by ChIP and processed into a library. Illumina HiSeq2000 was used for sequencing and non-redundant reads of 18772477 were obtained and processed for analysis (SICER). The data are deposited to Gene Expression Omnibus database with the accession number GSE51531 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51531). ChIP-quantitative PCR (qPCR) was performed from chromatin prepared similarly as ChIP-seq using anti-p300 Ab (sc-585; Santa Cruz Biotechnology) and anti-STAT1 Ab (sc-592). SYBR green–based qPCR was performed with custom designed primers as follows: Bcl6 forward, 5′-CTA GCG TCT GAC CAG GCC TGA-3′; Bcl6 reverse, 5′-AGG TCA CGC TCA AGG TTT GC-3′; Pdx1 forward, 5′-CAG CAC AGA AAT GGA AAA AGA GTG T-3′; Pdx1 reverse, 5′-CAG GCC CCA TGC TGA GAC T-3′; Cxcr5 forward, 5′-GGA TCA GGT TTT TAT CCG ATG AG-3′; and Cxcr5 reverse, 5′-TGC CCC ACG TTG TCT CTT-3′.

Statistical analysis

Statistical significance was determined by ANOVA using Prism software (GraphPad). A p value < 0.05 denoted the presence of a statistically significant difference.

Results

Type I IFNs promote sustained expression of Bcl6

To assess whether type I IFNs promote or inhibit TFH cell differentiation, we first tested the ability of these cytokines to induce Bcl6, the key transcription factor that influences specification of this subset of helper T cells. As shown in Fig. 1, naive CD4+ T cells activated by anti-CD3 and anti-CD28 and cultured in the absence of exogenous cytokines for 5 d expressed modest levels of Bcl6 and no T-bet, as judged by intracellular staining (Fig. 1A) or measurement of mRNA (Fig. 1B). Both IL-6 and IL-12 induced higher levels of expression of Bcl6, but the latter, as previously shown, also induced T-bet (49, 71). Interestingly, IFN-α and IFN-β both induced Bcl6 expression (Fig. 1A, 1B). Of note, there was no further induction of Bcl6 observed when type I IFNs were combined with IL-6 or IL-12 (Fig. 1C, 1D). IFN-α and IFN-β both induced T-bet expression, but neither was as effective as IL-12 in upregulating this transcription factor (Fig. 1A, 1B). Other transcription factors have been shown to play a role in TFH differentiation, including Maf, Irf-4, and Batf (72–75). Although IL-12 induced both Maf and Irf-4, IL-6 induced only Maf. In contrast, IFN-α and IFN-β had no effect on any of these transcription factors (Fig. 1B). Thus, type I IFNs promote Bcl6 expression, a key factor for Tfh cell function, and induce only low levels of T-bet.

Given that type I IFNs can drive both T-bet and Bcl6, and that these factors can be mutually antagonistic (49), we wondered if varying the dose of IFN-α or IFN-β would modulate the relative expression of these factors. Again, IFN-α and IFN-β induced T-bet expression in dose dependent manner (Fig. 1E). Surprisingly, Bcl6 expression also continued to rise when cells were stimulated with IFN-α and IFN-β. However, the induction of Bcl6 was much greater than that of T-bet (~80 versus 40%). Therefore, unlike IL-12, type I IFNs preferentially induced Bcl6 rather than T-bet.

Initially, IL-12 induces both Bcl6 and T-bet, but with time, T-bet expression overrides Bcl6 expression (49). To better understand the kinetics of these two factors in response to type I IFNs, we assessed their expression in the absence of exogenous cytokines or in the presence of IFN-β. Consistent with our previous findings, TCR- and CD28-mediated signals were sufficient to induce expression of Bcl6 in the majority of activated CD4+ T cells (Fig. 1F), but expression was transient and declined by day 5. In contrast, in the presence of IFN-β, Bcl6 expression was sustained. Thus, type I IFNs appeared to be positive regulators of Tfh differentiation, via induction of the master regulator responsible for this functionality.

Type I IFNs induce key TFH cell surface molecules

Having shown that type I IFNs induce the expression of a key transcription factor for Tfh cells, we next set out to determine whether these cytokines would induce other aspects of the genetic program of these cells. CXCR5 and PD-1 are key surface molecules expressed on Tfh cells that not only define this subset of cells but also are functionally relevant (8, 22–24, 76). We assessed whether all or some of these molecules were upregulated after treatment with type I IFNs. In the absence of exogenous cytokines, activated CD4+ T cells expressed little PD-1 and CXCR5 (Fig. 2A). With IL-6 treatment, very few cells became CXCR5+ PD-1+, and as previously shown, IL-12 was more effective in inducing expression of these molecules. The effects of IFN-α or IFN-β were more pronounced than either IL-6 or IL-12, as they induced a roughly 5 fold increase in the percentage of cells expressing CXCR5 and PD-1 compared with cultures in which no cytokine was added (Fig. 2A). Like Bcl6, increasing doses of IFN-α or IFN-β resulted in more cells expressing CXCR5 and PD-1 (Fig. 2B). We also assessed the time course of CXCR5 and PD-1 induction in the absence of cytokines, and after treatment with IFN-β. Not surprisingly, at day 3, the percentage of cells expressing CXCR5 and PD-1 was similar in both conditions, because these molecules are induced by general T cell stimulation (77, 78). By day 5, only cells treated with IFN-β continued to express these molecules (Fig. 2C), suggesting type I IFN drives and maintains both Bcl6 and the defining Tfh cell surface markers CXCR5 and PD-1.

Unlike its effects on Bcl6 expression, addition of IL-6 or IL-12 to IFN-α/β had an inhibitory effect on the percentage of cells expressing CXCR5 and PD-1 (Fig. 2D). Bcl6 upregulation typically parallels an increase in CXCR5 and PD-1, as was seen with IFN-α/β treatment alone; however, the addition of IL-6 or IL-12 to IFN-α or IFN-β resulted in sustained levels of Bcl6, but reduced expression of CXCR5 and PD-1.

ICOS is another key surface molecule for Tfh cells. It is required for Tfh cell development, and sustained expression of ICOS is an important feature of Tfh cells (4). We therefore also explored the effects of type I IFNs on the expression of ICOS. Unlike CXCR5 and PD-1, ICOS levels were highly expressed in all conditions (Fig. 2F). This suggests the regulation of ICOS is independent from the regulation of Bcl6, CXCR5, and PD-1.

Type I IFNs fail to induce IL-21

IL-21 is the “signature” cytokine for Tfh cells; although, the expression of this cytokine is not limited to Tfh cells and Tfh cells also make other cytokines (4). Given that IFN-α or IFN-β induced other key features of Tfh cells, we next asked whether treatment with IFN-α or IFN-β could promote IL-21 production. As expected, IL-6 was a very potent inducer of IL-21 (Fig. 3A, 3C) (32, 33, 79). Consistent with previous work, IL-12 also induced IL-21, but was less effective than IL-6 (Fig. 3A, 3C) (49). Despite their ability to induce Bcl6, CXCR5, and PD-1, IFN-α or IFN-β failed to robustly induce IL-21 at any point after culture with IFN-β, regardless of the dose used (Fig. 3A, 3C, 3D, 3E), or when subsequently cocultured with B cells (Fig. 3F, 3G) (80, 81). Higher doses of IFN-α/β increased the levels of IFN-γ production (Fig. 3D), paralleling its ability to induce T-bet (Fig. 1E). Thus, IFN-α/β...
did not efficiently induce IL-21, despite the induction of other TFH cell features, namely Bcl6, CXCR5 and PD-1. This was consistent with the inability of IFN-α/β alone to induce Maf, an important transcription factor involved in IL-21 production (Fig. 1B) (72). Of particular interest though, type I IFNs did synergize with IL-6 to induce IL-21; however, this was not the case for IL-12 (Fig. 3B, 3C). Collectively, these data support the notion that type I IFNs can promote some aspects of the TFH cell program, but are not sufficient to induce the entire TFH cell phenotype.

Divergent STAT requirements for Bcl6 and T-bet regulation

STAT3 is a contributing factor in TFH cells but unlike other subsets, its function appears to be partly redundant. STAT4 also promotes TFH cell differentiation mediated by IL-12. Type I IFNs activate STAT1, but can also activate STAT3 and STAT4 (54, 58). To ad-
dress the mechanism by which IFN-α or IFN-β support T<sub>FH</sub> cell programs, we first assessed STAT activation in naive CD4<sup>+</sup> T cells after treatment with type I IFNs. Whereas IL-6 induced phosphorylation of both STAT3 and STAT1, type I IFNs predominantly induced STAT1 activation (Fig. 4A, 4B). Under these circumstances, there was little STAT4 phosphorylation induced by any of these cytokines. Naive T cells do not express IL-12Rs, so the effects of this cytokine were not examined (82).

To determine which STAT, if any, was responsible for driving IFN-α– or IFN-β–induced T<sub>FH</sub> cell features, we isolated naive
CD4 T cells from wildtype, STAT1-, STAT3-, or STAT4-deficient mice and assessed the ability of IFN-α or IFN-β to induce T<sub>FH</sub> cell characteristics. As expected, expression of Bcl6 increased after culture with IFN-α or IFN-β (Fig. 4C). As type I IFNs did not induce STAT3 activation, this transcription factor was not necessary for Bcl6 induction. Similarly, in the absence of STAT4 the regulation of Bcl6 also was unaffected (Fig. 4C). In contrast, the induction of Bcl6 was markedly reduced in cells lacking STAT1.

FIGURE 3. Type I IFNs do not induce secretion of IL-21. (A–E) IL-21 and IFN-γ staining in CD4<sup>+</sup> T cells cultured in the presence of the indicated cytokines for 5 d (A, B), with increasing doses of IFN-α or IFN-β for 5 d (D) or in neutral conditions or IFN-β for the indicated number of days (E). Data (mean ± SD) are from duplicate cultures of three independent experiments (n = 6). The plots are representative of three independent experiments. *p < 0.05, **p < 0.01 (C–E). (F and G) Intracellular cytokine staining of IL-21 and IFN-γ in T cells cocultured with naive B cells for 4 d either with (stimulated) or without (unstimulated) anti-CD3 and anti-CD28 stimulation.
compared with cells expressing this factor, arguing that STAT1 is a major factor that drives Bcl6 levels in response to type I IFNs (Fig. 4C).

Interestingly, we found a different STAT is important for regulating T-bet. Expression of T-bet was minimal in cells lacking STAT4, despite stimulation with type I IFNs (Fig. 4C). IFN-α/β
also failed to induce IFN-γ production in STAT4-deficient cells (Fig. 4D). Thus, type I IFNs promote Bcl6 via STAT1 while promoting T-bet and IFN-γ via STAT4.

We also asked whether the regulation of CXCR5 and PD-1 by type I IFNs has similar requirements to Bcl6. Like Bcl6, upregulation of CXCR5 and PD-1 was dependent on STAT1 but not STAT3 or STAT4 (Fig. 4E). Collectively, these data suggest that type I IFNs largely exert their effect on Bcl6, CXCR5, and PD-1 via STAT1.

**STAT1 directly regulates key TFH cell genes**

Because our data indicated that the ability of type I IFNs to regulate critical TFH cell genes was STAT1 dependent, we next investigated whether this factor bound these genes directly. To this end, we performed STAT1 ChIP-seq analysis after treatment of naive CD4+ T cells with IFN-β and assessed the genome-wide binding of this factor. A total of 17635 STAT1 binding peaks were identified after IFN-β treatment. Consistent with the importance of STAT1 in regulating Bcl6 expression, we identified a strong area of STAT1 binding in the promoter region of Bcl6 (Fig. 5A). In addition, we found multiple sites of STAT1 binding upstream of the Bcl6 gene. Because there are no other genes in proximity, it is possible that these are enhancer elements and may be relevant for Bcl6 expression. Collectively, these data suggest that STAT1 might control Bcl6 by acting on its promoter but also distal enhancers. In addition to Bcl6, we also found binding of STAT1 upstream of Cxcr5 and Pdcd1 loci (Fig. 5A). The binding of the acetyltransferase p300 is a mark of active enhancers (83). We have previously shown that associated binding of p300 and STAT proteins is indicative of genomic regulatory enhancer elements generated by STATs, which can be associated with transcriptional activity (84). To determine whether STAT1 binding detected on TFH genes is functionally relevant, we performed ChIP-qPCR for p300 (Fig. 5B) and STAT1 reduced in STAT1-deficient cells, suggesting that STAT1 positively regulates the transcription of these TFH genes and that these are enhancer elements and may be relevant for Bcl6 expression. Upon binding to Bcl6, the STAT1 complex binds to the p300 acetyltransferase, which is a mark of active enhancers. Further studies are required to elucidate the functional role of the STAT1 complex in regulating Bcl6 expression in THF cells.

**Discussion**

Type I IFNs are highly expressed early during infections, but the role these factors play on CD4+ T cell differentiation remains unclear. In this study, we tested the role of IFN-α/β on TFH cell differentiation by exploring cardinal TFH cell features. IFN-α/β promoted several key TFH features, such as Bcl6, CXCR5, and PD-1 expression, and this effect was STAT1 dependent. Using ChIP-seq analysis, we found several STAT1 binding sites in the Bcl6, CXCR5, and Pdcd1 loci. At the Bcl6 loci, we found binding at both the promoter and potential enhancer regions, and these sites colocalized with p300 binding, a mark of active enhancers. These findings identify a new role for type I IFNs in controlling the expression of several genes involved in the TFH cell signature.

During an in vivo infection or immunization, multiple cytokines are induced, and each cytokine can activate more than one STAT transcription factor. Previous work from our group and others demonstrated that both STAT3 and STAT4 can promote TFH cell development, but this is not the case in vivo, neither of these factors is exclusively required, suggesting redundancy in the pathway to TFH cell generation (32–34, 49). Our results from this study demonstrate that STAT1, in addition to STAT3 and STAT4, can promote TFH cells.
responses. In addition, patients with SLE and a variety of other autoimmune diseases have a type I IFN signature that correlates with disease activity, suggesting constant exposure to these cytokines is a driver of disease (90). Our data suggest that IFN-α/β may be capable of supporting TFH cells, and this support could play an important role in autoantibody-driven autoimmune diseases.

Our observation that STAT1 binds to both promoter and potential enhancer elements of the Bcl6 loci suggest that STATs may play a critical and direct role in regulating Bcl6 expression. Because the binding motif for STATs is the same for all the STAT members except STAT6, we hypothesize that many of these potential enhancer sites can be occupied by STAT1, STAT3, STAT4, or STAT5. During an infection where multiple cytokines activate multiple STATS, it is easy to understand why several pathways can contribute to TFH cell differentiation both in vitro and in vivo. In the future, it will be important to carefully map all these sites throughout the extended Bcl6 locus and clarify their functional relevance. Clearly understanding the positive and negative roles of the STAT family and other transcription factors and their effects on the extended Bcl6 gene will be of considerable interest.

Acknowledgments

We thank G. Gutierrez-Cruz (Biodata Mining Core Facility, National Institute of Arthritis and Musculoskeletal and Skin Diseases), J. Simone, and


63. Ramos, H. J., A. M. Davis, T. C. George, and J. D. Farrar. 2007. IFN-
56. Teijaro, J. R., C. Ng, A. M. Lee, B. M. Sullivan, K. C. Sheehan, M. Welch,
2166 TYPE I IFNs PROMOTE Bcl6 INDUCTION VIA STAT1
59. Farrar, J. D., J. D. Smith, T. L. Murphy, and K. M. Murphy. 2000. Recruitment of
53. Ballesteros-Tato, A., B. León, B. A. Graf, A. Moquin, P. S. Adams, F. E. Land,
52. Nurieva, R. I., A. Podd, Y. Chen, A. M. Alekseev, M. Yu, X. Qi, H. Huang,
51. Ballestero-Tato, A., B. León, B. A. Graf, A. Moquin, P. S. Adams, F. E. Land,