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Bcl6 and Maf Cooperate To Instruct Human Follicular Helper CD4 T Cell Differentiation

Mark A. Kroenke,* Danelle Eto,* Michela Locci,* Michael Cho,† Terence Davidson,‡ Elias K. Haddad,§ and Shane Crotty*†

Follicular helper CD4 T (Tfh) cells provide B cells with signals that are important for the generation of high-affinity Abs and immunological memory and, therefore, are critical for the protective immunity elicited by most human vaccines. Transcriptional regulators of human Tfh cell differentiation are poorly understood. In this article, we demonstrate that Bcl6 controls specific gene modules for human Tfh cell differentiation. The introduction of Bcl6 expression in primary human CD4 T cells resulted in the regulation of a core set of migration genes that enable trafficking to germinal centers: CXCR4, CXCR5, CCR7, and EBI2. Bcl6 expression also induced a module of protein expression critical for T–B interactions, including SAP, CD40L, PD-1, ICOS, and CXCL13. This constitutes direct evidence for Bcl6 control of most of these functions and includes three genes known to be loci of severe human genetic immunodeficiencies (CD40L, SH2D1A, and ICOS). Introduction of Bcl6 did not alter the expression of IL-21 or IL-4, the primary cytokines of human Tfh cells. We show in this article that introduction of Maf (c-Maf) does induce the capacity to express IL-21. Surprisingly, Maf also induced CXCR5 expression. Coexpression of Bcl6 and Maf revealed that Bcl6 and Maf cooperate in the induction of CXCR4, PD-1, and ICOS. Altogether, these findings reveal that Bcl6 and Maf collaborate to orchestrate a suite of genes that define core characteristics of human Tfh cell biology. The Journal of Immunology, 2012, 188: 3734–3744.

Materials and Methods

Human samples

Fresh human tonsils were obtained from the University of San Diego Hillcrest Medical Center, the National Disease Resource Interchange, or the Children’s Hospital of Orange County. The majority of tonsils were from adults. Informed consent was obtained from all donors. Tonsils were homogenized using wire mesh and passed through a cell strainer to make a single-cell suspension. Mononuclear cells were isolated using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO). All protocols were approved by the La Jolla Institute for Allergy and Immunology (LIAI) and National Disease Resource Interchange, LIAI and University of San Diego, or LIAI and Children’s Hospital of Orange County Institutional Review Boards.

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Flow cytometry and sorting

For intracellular staining, cells were restimulated with 25 ng/ml PMA and 1 μg/ml ionomycin in the presence of 10 μg/ml brefeldin A for 3 h. Cells were fixed with BD Phosflow Perm Fix Buffer 1 and permeabilized with BD Phosflow Perm/wash Buffer 1.

All cells were sorted using a BD FACSAria. All Tfh cell sorts were initially gated on CD4+CD19+ 7-AAD- cells. CD45RO- cells were confirmed to be naive by costaining for CD45RA. Naive B cells were CD19+ CD20+CD3- CD27 IgD-. The following anti-human Abs were used: CD45RO (clone UCHL1), CD45RA (clone HI100), CD19 (clone HIB19), CD38 (clone HIT2), CD20 (clone 2H7), CD27 (clone O23E), PD-1 (clone J105), ICOS (clone ISA-3), CXCR4 (clone 12D6), CD40L (clone 24-31), CD40 (clone 40B4), CD4 (clone RPA-T4) (eBioscience, San Diego, CA); CXCR5 (clone RF8B2), CCR7 (clone 3D12), Bcl6 (clone K112-91), and IgD (clone IA6-2) (BD Biosciences, Franklin Lakes, NJ); and SAP (clone 1D12) (Cell Signaling Technology, Danvers, MA) (21). For intracellular staining, the following Abs were used: IL-4 (clone MP4-25D2; BioLegend, San Diego, CA) (21). For intracellular cytokine staining done in transduced cells (Fig. 6), a two-tailed, paired test was used. For ectopic expression of Maf + Bc6, two-tailed paired t tests were used. For correlative analyses between Bc6 and other markers, linear-regression analysis was performed, and R2 was calculated using Prism 5.0 (GraphPad, La Jolla, CA).

Results

Bcl6 protein is expressed by human Tfh and GC Tfh cells

Bcl6 mRNA is present in CXCR5+ CD4 T cells isolated from human tonsil (18, 25, 26). However, Bcl6 mRNA levels frequently do not correlate with Bc6 protein expression (13). Bc6 protein has been detected in human CD4 T cells by immunofluorescence (26, 27). To address the importance of Bc6 in human Tfh cell differentiation, we first examined Bc6 protein expression in human CD4 T cells at the single-cell level by flow cytometry. Human tonsil is a lymphoid tissue rich in GCs. When tonsillar B cells were stained with a Bc6 mAb, expression was limited to CD20+CD38+ GC B cells, as expected (Supplemental Fig. 1). Tonsillar CD4 T cells were gated on CD45RO- (memory/effector) or CD45RO+ (naive), and CD45RO- were subsequently divided into CXCR5int, CXCR5hi, and CXCR5- gates (Fig. 1A). At least two distinct populations of CXCR5-expressing Tfh cells exist: one population within the GC and another population outside the GC (1, 28). In mice, these two populations have been termed GC Tfh and Tfh cells, respectively, and they can be distinguished on the basis of PD-1 expression (29). Given the dynamic nature of CD4 T cells entering and exiting GCs (30, 31), it is likely that Tfh and GC Tfh cells represent two interconverting activation states of the same cell type captured at different moments in time (1). Although human GC Tfh cells are sometimes defined by CD357 expression (32, 33), CD57 is not exclusively expressed on GC Tfh cells (25, 34). These CXCR5int Tfh and CXCR5hi GC Tfh cell populations are more precisely delineated by co-staining with ICOS (34, 35) (Fig. 1B) or by their dramatically high level of PD-1 (18, 25) (Fig. 1A). We found that both CXCR5int and CXCR5hi cells expressed significantly more Bc6 protein compared with CXCR5- CD45RO+ cells (p = 0.0014 and 0.0038, Fig. 1B, 1C). CXCR5hi cells also expressed very high levels of PD-1, ICOS, CXCR4, and SLAM-associated protein (SAP) compared with CXCR5int or CXCR5- cells (Fig. 1B, 1C). CXCR5int Tfh cells also express most canonical Tfh cell markers but at lower levels than CXCR5hi cells (Fig. 1B, 1C). We further characterized the different tonsillar CD4 T cell subsets by comparing their B cell help capabilities in coculture with B cells. When cultured in the presence of autologous naive B cells and SEB, both CXCR5hi and CXCR5hi CD4 T cells induced substantial IgM production (Fig. 1D), consistent with both CXCR5int and CXCR5hi CD4 T cells being related populations of Tfh cells. The majority of the Ab secretion was dependent on IL-21 (Fig. 1D), consistent with previous reports (25, 35, 36).

Bc6 instructs the conversion of Tfh cells to GC Tfh cells

To determine whether high Bc6 expression was sufficient to induce the human GC Tfh cell phenotype, we constructed a lentiviral expression vector, Bc6-LV, that constitutively expresses Bc6 and a fluorescent protein (ZsGreen). CXCR5hi Tfh cells were sorted from tonsil (Fig. 2A), stimulated with anti-CD3/CD28-coated beads,
and transduced with either Bcl6-expressing viral vector (Bcl6-LV) or an equivalent vector with no Bcl6 insert (Ctrl-LV) (Fig. 2B). Bcl6-LV drove a nearly 10-fold increase in Bcl6 protein expression compared with Ctrl-LV (Fig. 2C). Importantly, this increase in Bcl6 expression led to strong upregulation of CXCR5, the central marker of Tfh cells ($p = 0.0011$, Fig. 2D). The mean fluorescence intensity (MFI) of CXCR5 exhibited a strikingly strong linear relationship with the MFI of Bcl6 ($R^2 = 0.97$) (Fig. 2K).

Bcl6 expression also drove the expression of another major chemokine receptor, CXCR4 ($p = 0.0016$) (Fig. 2E). Detailed analysis revealed that CXCR4 levels exhibited a tight linear correlation with Bcl6 expression ($R^2 = 0.96$, Fig. 2K). Cells that expressed intermediate levels of ZsGreen and Bcl6 exhibited the same changes as the top 5–10% but to a lesser extent (Supplementary Fig. 2). CCR7 was consistently downregulated in the presence of Bcl6 ($p = 0.0172$, Fig. 2F; $R^2 = 0.81$, Fig. 2L). Downregulation of CCR7 is critical for T cell entry into the B cell follicle (37). Bcl6 also negatively regulated the G protein-coupled receptor EBI2 (Fig. 2M, $p = 0.004$). EBI2 downregulation is critical for movement of B cells from the outer follicle into the GC (38–41), but there is no known role for EBI2 in CD4 T cells. In this study, we show that Bcl6 mediates repression of EBI2, which is likely important for entry of Tfh cells into the GC. CXCR4 expression is important for correct GC architecture and is associated with localization of GC B cells to the dark zone (42). Therefore, Bcl6 regulates the migratory capacity of human Tfh cells for appropriate localization to, and within, GCs by controlling CXCR5, CXCR4, EBI2, and CCR7 expression.
SAP is an intracellular SH2 domain-signaling molecule that transmits signals for SLAM family receptors, and mutations in the gene encoding SAP (SH2D1A) are the cause of the severe human genetic immunodeficiency X-linked lymphoproliferative disease (43). SAP is critically required for GC and memory B cell development in both mice and man (30, 44, 45), and murine GC Tfh cells require SAP expression (29). No connection has been made between Bcl6 and SAP. Therefore, we examined whether SAP expression is regulated by Bcl6 in human CD4 T cells. SAP was uniformly upregulated by Bcl6 expression (p = 0.0002, Fig. 2G). SAP mRNA was also upregulated in Bcl6-expressing cells (data not shown). The data in this study now directly connect these two central regulators of T cell help to B cells.

In addition to Bcl6 directing the upregulation of SAP, the cell surface signaling molecule PD-1 was upregulated by Bcl6 (p = 0.0013, Fig. 2H). PD-1 expression by Tfh cells may be important for regulating Tfh cell proliferation and signaling to GC B cells. ICOS is also central to T–B interactions with B cells. Humans with ICOS deficiencies exhibit severe Tfh cell and GC defects and have minimal response to vaccines (46–48). Tfh cells depend on ICOS ligand signals from B cells to maintain Bcl6 expression (49). Therefore, we examined whether ICOS expression is regulated by Bcl6 in human CD4 T cells. ICOS expression was modestly increased by expression of Bcl6 (p = 0.04, Fig. 2I).

Surprisingly, CD40L was upregulated by expression of Bcl6 (p = 0.04) (Fig. 2J). CD40L is one of the most critical molecules for T cell help to B cells. Mice deficient in CD40-CD40L signaling have neither GCs nor plasma cells (50–55). Defective CD40–CD40L signaling in humans causes the severe immunodeficiency hyper-IgM syndrome (56). Therefore, human Bcl6 controls both the central components of Tfh cell migration (CXCR5, CXCR4, CCR7, EBI2) and multiple central components of T–B interactions (SAP, PD-1, ICOS, CD40L). Collectively, these data indicate that Bcl6 instructs Tfh cell differentiation by regulating a program of gene expression that allows the T cells to colocalize with B cells and provide critical help functions.

Bcl6 can convert non-Tfh cells to Tfh cells

Next, we set out to determine whether Bcl6 expression was sufficient to drive the differentiation of effector CD4 T cells.
Bcl6 and Maf instruct human Tfh differentiation

**FIGURE 3.** Bcl6 converts human CXCR5^+ CD4 T cells to Tfh cells. (A) CD45RO^+CXCR5^+ CD4 T cells were sorted from human tonsil. (B) Expression of Bcl6 was measured based on the top gates shown in Supplemental Fig. 2A. (C-I) Expression of Tfh cell-associated molecules by Bcl6-LV and Control-LV (Ctrl-LV) groups based on gates used in Supplemental Fig. 2A. A representative plot is shown (left panel) alongside the complete data set with five or six individual donors (right panel). (J) ZsGreen-expressing cells were divided into five gates based on ZsGreen MFI. Bcl6, CXCR5, CXCR4, PD-1 (J), and CCR7 (K) MFIs were determined for each ZsGreen gate. Data shown in (J) and (K) are representative of six individual donors. *p < 0.05, **p < 0.005.

**FIGURE 4.** Bcl6 instructs CXCL13 production by human Tfh cells. (A) Selective production of CXCL13 by human Tfh cells. Unstimulated tonsil cells were incubated for 3 h with brefeldin A before intracellular staining for CXCL13. Left panel, CXCL13 expression shown in total CD4 T cells. Activated CD4 T cell subsets were gated (middle panel), and CXCL13 levels for each population are shown versus isotype control (right panel). Isotype control was gated on total CD4 T cells. MFI for each population is indicated. (B) CXCR5^+ Tfh cells were sorted from tonsil, stimulated with anti-CD3/CD28 beads, and transduced with Bcl6-LV or Control-LV. Five days posttransduction, CXCL13 present in the supernatant was analyzed by ELISA. Data are representative of four total donors from two independent experiments. *p < 0.05, **p < 0.01.
Nevertheless, expression of Bcl6 in human CXCR5<sup>−</sup> effector Th cells led to a much greater and sustained increase in CXCR5 expression \( (p = 0.0099, \text{Fig. 3C}) \). Furthermore, Bcl6<sup>+</sup> cells specifically exhibited significantly increased expression of SAP, PD-1, and CD40L \( (p = 0.001, 0.002, \text{and} 0.02, \text{respectively, Fig. 3F, 3G, 3I}) \), as well as downregulation of CCR7 \( (p = 0.01, \text{Fig. 3E;} R^2 = 0.80, \text{Fig. 3K}) \). A small increase in ICOS was observed \( (\text{Fig. 3H,} p < 0.05) \). A robust correlation between Bcl6 and both CXCR5 and CXCR4 expression was observed \( (R^2 = 0.88 \text{and} 0.97, \text{Fig. 3J}) \). Similar effects were seen when starting with naive (CD45RO<sup>−</sup>CXCR5<sup>−</sup>) CD4 T cells (Supplemental Fig. 4). Overall, these data demonstrate that Bcl6 controls a program of Thf cell gene expression in human CD4 T cells, and Bcl6 expression is sufficient to convert previously Ag-experienced human CD45RO<sup>+</sup>CXCR5<sup>−</sup> CD4 T cells into Thf cells.

### Bcl6 induces CXCL13 production

Thf cells are well known as important producers of the helper cytokines IL-4 and IL-21 \( (1) \). Human Thf cells, but not murine, also specifically express the chemokine CXCL13 \( (32, 34, 36) \), a B cell-attracting molecule usually made by stromal cells \( (59) \). We hypothesized that CXCL13 expression by Thf cells may be regulated by Bcl6. Unmanipulated human GC Thf cells are a significant source of CXCL13 protein, as determined at the single-cell level (Fig. 4A). Impressively, when Bcl6 was constitutively expressed in human CXCR5<sup>+</sup> Thf cells, thereby inducing a GC Thf cell phenotype (Fig. 2), >10-fold increases in CXCL13 protein were detected in culture supernatants compared with control cells \( (60–100 \text{ pg/ml,} p < 0.05, \text{Fig. 4B}) \). CXCL13 mRNA was also upregulated in Bcl6-expressing cells (data not shown).
cialized production of CXCL13 by GC Tfh cells may help to properly organize the GC and may also provide cytokine-like signals to GC B cells.

**Helper cytokines IL-4 and IL-21**

A population of human GC Tfh cells produce both IL-4 and IL-21 (Fig. 5). These cytokines have been implicated in the maintenance of GC B cells and the differentiation of long-lived plasma cells (1, 60). IL-4 is known to provide prosurvival signals to B cells (61, 62). Mice dually deficient in both IL-21 and IL-4 signals had significantly reduced B cell responses (63). Human GC Tfh cells exhibited considerably more IL-4 expression than did Tfh cells or even the effector CD4 T cell (effector Th) population that includes Th2 cells (Fig. 5). Virtually all of the GC Tfh IL-4+ cells also expressed IL-21, the canonical Tfh cell cytokine (Fig. 5) (6, 26, 49, 64). The frequency of IL-4+IL-21+ cells in the CXCR5hi GC Tfh cell population was significantly higher than in the CXCR5int cell population (p = 0.005) or the effector Th subsets (p = 0.003, Fig. 5).

The Tfh cell program is perhaps best conceptualized as a developmental program that can coexist with Th1, Th2, or Th17 gene expression to a limited extent (1). Therefore, we examined the capacity of human Tfh and GC Tfh cells to produce Th1 or Th17 cytokines. GC Tfh cells secreted significantly less IL-17 compared with Tfh or effector Th cells (Fig. 5). A modest reduction in IFN-γ+ cells was observed for GC Tfh cells compared with effector Th cells (p = 0.225 and 0.239, respectively) (Fig. 5). A considerable percentage of human CXCR5int Tfh cells produced IL-17 or double produced IFN-γ and IL-17, consistent with CXCR5int Tfh cells being a less polarized and more heterogeneous cell type than CXCR5hi GC Tfh cells (Fig. 5).

Given that Bcl6 controls both Tfh cell migration and Tfh:B interaction genes, we set out to determine whether Bcl6 also instructs GC Tfh cell cytokine expression. Unexpectedly, constitutive expression of Bcl6 did not significantly increase expression of IL-4 or IL-21 in any CD4 T cell subset examined (Fig. 6). Furthermore, the IL-4+/IL-21+ population characteristic of GC Tfh cells was not observed (Fig. 6). Therefore, although Bcl6 instructs many aspects of the human Tfh cell phenotype, the production of B cell help cytokines requires additional signals.

**Maf induces IL-21 secretion in CD4 T cells**

Maf is a transcription factor involved in IL-21 (65, 66) and IL-4 (67) production in mouse CD4 T cells. Maf is expressed at a higher level in CXCR5hi and CXCR5int human Tfh cells than in naive or CXCR5−CD45RO− cells (Fig. 7A). Maf expression can be induced by ICOS signaling (68, 69). Consequently, we set out to test the hypothesis that Maf contributes a complementary role to Bcl6 in Tfh cell differentiation by inducing secretion of helper cytokines. To test this hypothesis, we generated a viral expression vector containing human Maf. Transduced cells were visualized by their expression of ZsGreen (Maf) or a truncated, nonsignaling human nerve growth factor receptor (dNGFR; Bcl6). Transduction of CD45RO+CXCR5+CD4 T cells with Maf did not result in a notable change in the percentage of cells secreting IL-4 upon restimulation (Supplemental Fig. 3). However, Maf expression...
resulted in a marked increase in the percentage of IL-21–pro-
ducing CD4 T cells compared with control-transduced or Bcl6-
transduced cells (\(p = 0.0009\) and 0.016, respectively, Fig. 7B, 7C).
Dual transduction with both Maf and Bcl6 (Fig. 7D) did not in-
crease IL-21 production above the percentage induced by Maf
alone (Fig. 7C). Dual transduction with both Maf and Bcl6 did not
enhance IL-4 production (Supplemental Fig. 3). Maf is expressed
by Th17 cells (65, 69); however, no significant change in IL-17
expression was observed in Maf-transduced human CD45RO+
CXCR5int CD4 cells (data not shown). Naive CD4 T cells that
were activated and then transduced with Maf also did not express
IL-17 (data not shown). These data demonstrate that, although
Bcl6 is critical for T cell positioning within the follicle and direct
T–B interactions, Maf, but not Bcl6, can enhance IL-21 produc-
tion by CD4 T cells.

**Maf cooperates with Bcl6 to instruct human Tfh cell differen-
tiation**

We examined whether Maf regulates additional Tfh cell-associ-
ated proteins. Surprisingly, Maf induced a significant increase in
CXCR5 expression, and this effect was particularly notable when
starting with CD45RO+ CXCR5– naive CD4 T cells (\(p = 0.0002\),
Fig. 8A, 8B). This was not due to Maf induction of Bcl6 (Fig. 8C).
Of note, ectopic expression of Bcl6 induced a modest, but sig-
nificant, increase in Maf expression (Fig. 8D). Cotransduction of
naive CD4 T cells with Bcl6 and Maf did not further enhance
CXCR5 expression. In contrast, ICOS and PD-1 expression both
increased significantly when Bcl6 and Maf were ectopically
expressed together (Fig. 8E, 8F). CXCR4 was also significantly
induced by Maf at levels comparable to that seen with Bcl6, and
further increased CXCR4 levels were observed in doubly trans-
duced cells (\(p = 0.03\), Fig. 8G). Unlike Bcl6, Maf did not have an
impact on CD40L expression (Fig. 8H). Taken together, these
data show that Bcl6 and Maf both play a role in human Tfh cell
differentiation. Bcl6 controls modules of genes important for Tfh
cell localization and T–B interactions. Complementing Bcl6, Maf
induces IL-21 secretion and CXCR5 expression and enhances ex-
pression of a number of Bcl6-regulated Tfh cell-associated mole-
cules, such as ICOS and PD-1.

**Discussion**

In-depth knowledge of the pathways of human CD4 T cell differen-
tiation is critical for understanding how to accomplish rational
vaccine design against the myriad pathogens that humans remain
unprotected against. In addition, understanding human CD4 T cell
differentiation is critical for understanding most human autoim-
une diseases. These feats can only be accomplished with a clear
understanding of the transcription factors that regulate human CD4
T cell differentiation. In this study, we demonstrate that Bcl6
is a central regulator of human Tfh cell differentiation, and we
demonstrate that Bcl6 accomplishes this task by controlling two
major modules of Tfh cell gene expression. In human B cells, Bcl6
was generally shown to repress gene transcription (14, 15). It is
likely that upregulation of many Tfh cell genes is an indirect effect
brought about by Bcl6-mediated repression of specific transcription
factors and chromatin remodelers, such as histone-modification
enzymes (9, 10). However, the possibility that Bcl6 binding to
some genes may positively influence gene expression must also
be considered.

There has been only a limited understanding of how Bcl6
controls CD4 T cells, and little data have been available on Bcl6
function in human CD4 T cells (18, 25). Ectopic expression of
Bcl6 in murine CD4 T cells in vivo induced a Tfh cell phenotype
But it had surprisingly limited activity in purified murine CD4 T cells in vitro, with minimal Tfh cell-associated protein changes (17, 18, 70). Importantly, our in vitro human CD4 T cell system allowed us to identify downstream targets of Bcl6 regulation, without the confounding effects of non-Bcl6 signals present in the in vivo mouse models that also contribute to Tfh cell differentiation. We demonstrate that introduction of Bcl6 into human CD45RO+ CD4 T cells converts those cells to a Tfh-like cell phenotype in vitro, and the degree of conversion strongly correlates with the level of Bcl6 expression (Figs. 2, 3). We show that Bcl6 regulates distinct modules of the Tfh cell program: one Bcl6-dependent module is genes critical for Tfh cell migration (CXCR5, CXCR4, CCR7, EBI2), and the second Bcl6-dependent module is a set of genes important for T–B interactions (SAP, PD-1, CD40L, ICOS, CXCL13), including two genes known to be critical for contact-dependent B cell help (SAP and CD40L). Therefore, Bcl6 is a true nexus for human Tfh cell differentiation and functions. Perhaps the most striking finding from this study is that Bcl6 specifically regulates CD40L, SAP, and ICOS. From the perspective of human immunology, the CD40L, SH2D1A, and ICOS genes are three loci of severe immunodeficiencies of adaptive immunity. Genetic lesions in CD40L and SH2D1A are lethal because of a resulting susceptibility to a range of infectious diseases. Extreme losses in responsiveness to vaccines and failure to develop B cell memory are prominent characteristics of these genetic diseases. Deletion of the human ICOS gene also results in immunodeficiency, susceptibility to infections, and a failure to respond to vaccines (46–48), consistent with the importance of ICOS for Tfh cell differentiation (49). In this study, we find that Bcl6 regulates all three of these critical human genes, highlighting its powerful role in defining Tfh cell functionality.

The data in this study also show that PD-1 is explicitly regulated by Bcl6. Therefore, the high level of PD-1 on Tfh cells is not simply a byproduct of TCR stimulation but is a specific component of the Tfh cell gene program. In the absence of PD-1 in mice, increased GC B cell death and a defective plasma cell response were observed in one study (71), whereas excessive Tfh cell proliferation was seen in another study (72). PD-1 is a potent negative regulator of T cell proliferation. We propose that PD-1 is an important negative regulator of Tfh cells, most likely by dissociating Tfh cell TCR signaling from proliferation. The purpose of GCs is the rapid evolution of BCR affinity through rapid GC B cell proliferation and hypermutation. Tfh cells are critical for this process and must preferentially select the “best” GC B cells for further rounds of proliferation and mutation via sensing quantitative differences in peptide:MHC complexes between different GC B cells. This must require highly sensitive TCR signaling. At the same time, GC Tfh cell numbers remain relatively static for the duration of the GC. Therefore, Bcl6 induction of PD-1 on Tfh cells is likely
important for restriction of Thf cell proliferation in the presence of continuous Ag stimulation.

The role of Bcl6 in human Th cell differentiation differs from murine Th cell differentiation in several key ways. Direct induction of CXCR5 protein expression on purified CD4 T cells is one example. Bcl6 regulation of CXCL13 is a second example. CXCL13 is expressed by human Tfh cells but not mouse Th cells. Production of CXCL13 by Th cells may serve two purposes. First, CXCL13 is a chemoattractant and will recruit B cells to the location of the Tfh cells. This may be important in the face of significant reductions in CXCL13 expression by lymphoid tissue stromal cells during viral infections. (73). Second, CXCL13 binding to CXCR5 on B cells induces LTα1β2 expression (74). As such, Thf cell expression of CXCL13 may orchestrate GC architecture. This CXCL13 expression by Thf cells may also drive tertiary lymphoid neogenesis in autoimmune diseases (4). CXCL13 and LTα1β2 are important components of lymphoid organ neogenesis and the formation of ectopic lymphoid aggregates (75). It is feasible that CXCL13 binding to CXCR5 on GC B cells induces other nonchemotactic signaling responses that are important for GC B cell survival or differentiation.

IL-21 is the most important known cytokine for Thf cell help to B cells, both through maintenance of GCs and for induction of plasma cell differentiation. In this study, we found that Bcl6 has minimal influence on IL-21 production, whereas Maf does drive IL-21 production. More surprisingly, Maf also controls other components of human Thf cell function, including expression of CXCR5. Intriguingly, coexpression of Bcl6 and Maf exhibited additive effects on gene expression for some Thf cell-associated genes (e.g., PD-1 and ICOS), whereas, in other cases, Bcl6 and Maf appear to have parallel, nonadditive capacities (e.g., CXCR5). Altogether, these finding reveal that Bcl6 and Maf collaborate to orchestrate a suite of genes that define nearly all of the core characteristics of human Thf cell biology.

We demonstrated that Bcl6 and Maf synergistically induce human Thf cell differentiation and function. As such, manipulation of Bcl6 and/or Maf activity in a positive way in human CD4 T cells may have an important impact for human vaccines, resulting in enhanced Thf cell number or functionality and long-term B cell immunity. Alternatively, inhibition of Bcl6 and Maf in CD4 T cells is an outstanding candidate for ameliorating autoimmune diseases associated with autoantibody production.

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Disclosures

The authors have no financial conflicts of interest.

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