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New effector functions and regulatory mechanisms of BCL6 in normal and malignant lymphocytes

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Abstract

The BCL6 oncogenic repressor is a master regulator of humoral immunity and B-cell lymphoma survival. Whereas much research has focused on its regulation and function in germinal center B-cells, its role in other mature lymphoid cell compartments is less clear. A novel role for BCL6 in follicular T helper cell development was recently uncovered. The latest discoveries reveal that BCL6 is also an important regulator of other specialized helper T-cell subsets within germinal centers, pre-germinal center events, and peripheral T-cells effector functions. Here, we review newly discovered roles for BCL6 in lymphocyte subsets residing within and outside of germinal centers, and discuss their implications with respect to the molecular mechanisms of BCL6 regulation and potential links to B and T-cell lymphomas.

Introduction

BCL6 (B Cell Lymphoma 6) is a transcriptional repressor and member of the BTB/POZ (bric-à-brac, tramtrack, broad complex/pox virus zinc finger) family of transcription factors. The BCL6 gene was initially cloned by several groups in 1993 from a translocation occurring on chromosome 3q27 in diffuse large B-cell lymphoma (DLBCL) [1]. Targeted disruption of the *BCL6* gene revealed a critical role for BCL6 during normal B-cell development, as a master regulator of antibody affinity maturation in germinal centers (GCs) [2]. BCL6 is almost universally expressed in GC-derived B-cell lymphomas, including DLBCL and follicular lymphomas (FLs), regardless of translocations.

Beyond the mature GC B-cell population, novel roles for BCL6 have recently been uncovered in several different T lymphocyte subsets within the GC, including CD4+ follicular helper T (T_{FH}) cells, follicular regulatory T (T_{FR}) cells and follicular helper natural killer T (NKT_{FH}) cells. Newer studies have also revealed a much earlier involvement of BCL6 in pre-GC B and T-cell events. Outside of GCs, new roles for BCL6 have been identified in T regulatory (T_{reg}) cells and in the control of T-cell-dependent inflammation. These new studies point to a broader function of BCL6 both in the GC reaction as well as in T cell-dependent inflammation and autoimmunity.

These discoveries raise many new questions about the full spectrum and context specificity of BCL6 target genes and downstream mechanisms in these various cell types, as well as the molecular mechanisms underlying the regulation and dysregulation of the *BCL6* gene in normal and malignant lymphocytes. Recent advances in these topics will be discussed pertinent to the effector functions of normal B- and T-cells and in the development of B- and T-cell lymphomas.

Novel functions of BCL6 within the GC

While the importance of BCL6 in GC B-cell differentiation is well known [3,4], a role for BCL6 in GC T-cells was only recently discovered. Three independent studies identified a critical role for BCL6 in the differentiation of a specialized subset of CD4⁺ T_{FH} cells in the GC [5–7], which provide help to antigen-specific B-cells and are phenotypically distinguished by high expression of CXCR5, PD-1, IL-21, SAP, ICOS and BCL6 [8]. A primary function of BCL6 in T_{FH} cells appears to be the suppression of genes that are required to drive the differentiation of alternative T helper cell lineages. Competition between BCL6 and T-bet, a driver of T helper type-1 (Th1) cells, is important for determining the commitment of transitional T helper cells toward the T_{FH} phenotype [9,10]. However, the presence of these and other factors, such as IL-21 and IL-2, also enables T_{FH} cells to retain flexibility in their T helper lineage specification [9–11]. Remarkably, unlike GC-derived memory B-cells, which do not express BCL6 [12], BCL6⁺ effector T_{FH} cells are capable of forming follicular helper-like central memory cells [13–15], implicating BCL6 expression in long-lived T helper cell immunity. BCL6 was also identified recently in a new subset of follicular T regulatory (T_{reg}) cells that localize to GCs and express the T_{FH} markers, CXCR5 and PD-1, and the T_{reg} cell marker, FOXP3 [16,17]. T_{FR} cells, which originate from thymic FOXP3⁺ precursor cells, require BCL6 for their development and appear to control the magnitude of the GC response [18,19]. Yet another specialized subset of BCL6⁺ GC T cells, follicular helper natural killer T (NKT_{FH}) cells, were recently shown to provide direct help to antigen-specific B-cells; however, were unable to drive the generation of long-lived plasma cells [20]. Together with its functions in B-cells, BCL6 orchestrates a diverse sequence of T-cell subtypes (Figure 1a) to regulate B cell affinity maturation in the GC.

Pre-GC roles for BCL6 in B and T cells

Recent employment of BCL6-reporter mice and high-resolution intravital cellular imaging, enabling *in vivo* tracking of specific lymphocyte populations, have alluded to an even earlier role for BCL6 in pre-GC events [21,22] (Figure 1a). *In vivo* tracking of BCL6⁺ cells using YFP or RFP reporter systems have shown that BCL6 not only participates in the earliest phase of T_{FH} development [15,23,24], but is also up-regulated in antigen-engaged B cells prior to their clustering to form GC structures [25]. Using intravital imaging in mice, Kerfoot and colleagues independently demonstrated an early role for sustained *BCL6* expression during B and T_{FH} cell interactions within the interfollicular zone prior to GC entry [26]. Early B:T-cell interactions that lead to extrafollicular antibody responses, have also been shown to be dependent on SAP and IL-21 and on BCL6⁺ pre-GC T_{FH} cells (Figure 1a) that express low levels of PD-1 and are phenotypically distinct from T_{FH} cells in

the GC [27,28]. What causes *BCL6* to become up-regulated in B and T cells at this early stage of GC formation is not fully understood.

New roles for BCL6 outside of GCs

Several new roles for BCL6 in lymphocyte lineages that exist outside of GCs have been recently described (Figure 1b). *BCL6* is up-regulated upon pre-B cell receptor signaling in pre-B cells undergoing immunoglobulin V(D)J recombination, protecting cells from DNA-damage-induced apoptosis [29] and, subsequently, enabling cell cycle exit through repression of *MYC* and *CCND2* [30]. This late pre-B cell checkpoint function of BCL6 [30] could help to prevent malignant transformation of pre-B cells to acute lymphoblastic leukemia (ALL). However, BCL6 can also sustain the proliferation of established B-cell ALL (B-ALL), as well as offer a protective role against DNA-damaging classes of chemotherapeutics during B-ALL treatment [31,32]. Within the T-cell compartment, BCL6 was previously shown to be important for the generation and maintenance of effector and memory CD8⁺ T cells and memory CD4⁺ T cells [33]. Peripheral CD4⁺ T_{FH} cells (CXCR5^{lo}ICOS^{lo}IL-7R⁺) that express *BCL6* and are functionally similar to GC T_{FH} cells have recently been identified and provide help to B-cells outside of GCs [34]. Consistent with the severe T helper type-2 (Th2)-type inflammatory disease that develops in BCL6-deficient mice [35], BCL6 has been shown to control the effector functions of peripheral T_{reg} cells [36]. BCL6 also regulates the differentiation of T helper IL-17-expressing (Th17) cells, via both T-cell intrinsic and extrinsic mechanisms [5,6,37]; however, in the BCL6^{-/-} model of Th2 inflammation, increased Th17 cell activity appears to be mediated largely through the T-cell extrinsic function of BCL6 in macrophages [37]. Furthermore, *ex vivo* over-expression of *BCL6* in CD8⁺ T-cells enables conversion to CD8⁺ T cells with suppressor activity, which protect against T-cell-dependent autoimmunity [38]. These studies suggest that in addition to controlling chemokine expression in macrophages [39,40], BCL6 could help to control inflammation and autoimmunity by directly regulating the differentiation of Th17, T_{reg} and CD8⁺ T suppressor cells.

Transcriptional mechanism of action of BCL6 in normal and malignant lymphocytes

BCL6 mediates its actions in GC B-cells through the repression of genes (e.g. *ATR*, *TP53*, *CDKN1A*, *EP300*) to enable proliferation and survival during affinity maturation, and by repression of terminal differentiation factors (e.g. *IRF4*, *PRDM1*) [4,41]. The BCL6-driven gene expression programs of T-cells are less well characterized. In general, it appears that in CD4⁺ T-cells, BCL6 promotes the expression of genes that confer the GC T_{FH} cell phenotype (*CXCR5*, *CXCR4*, *PDI*) by repressing the transcription factors, *Tbx21* (T-bet), *Gata3* and *Rorγt*, to block interferon-gamma (IFNγ-, IL-4- and IL-17-mediated differentiation to the Th1, Th2 and Th17 cell lineages [5,6]. These events appear to be dependent on the DNA binding activity of BCL6 [6]. BCL6 also represses *GATA-3* expression in T_{reg} cells and during Th17 cell differentiation [36,37]. As seen in GC B-cells, BCL6-mediated repression of *Blimp-1* is required to maintain the T_{FH} phenotype [7,10]. In addition to suppressing T lineage-specific transcription factors, BCL6 directly represses several microRNAs, including *miR-17-92*, which down-regulates expression of *CXCR5* in

T_{FH} cells [5] and *miR-21*, which, via up-regulation of a MAPK pathway inhibitor, decreases *GATA-3* expression in T_{reg} cells [42]. Using BCL6-RFP reporter mice, Liu and colleagues showed that CD4⁺ T cells that express high (but not low) levels of BCL6 are distinguished by a T_{FH} cell-specific gene signature (*CXCR5*, *CXCR4*, *SAP*), but not by genes that are characteristic of Th1, Th2, Th17, or T_{reg} cell subsets [15]. Genome-wide maps of BCL6 binding in GC T_{FH} cells and other non-GC T-cell subsets, together with perturbation of BCL6 and gene expression profiling, will be critical to elucidate the programs of BCL6-mediated repression that specifically direct T-cell (versus B-cell) development and effector functions.

From the biochemical standpoint, BCL6 is believed to mediate its repressive effects largely by recruiting the transcriptional corepressors, silencing mediator for retinoid or thyroid-hormone receptors (SMRT), nuclear receptor corepressor-1 (NCOR) and BCL6-interacting corepressor (BCOR), through its N-terminal BTB domain [3]. SMRT and NCOR, in particular, are ubiquitously expressed and so would be expected to enable BCL6 functions regardless of cell context. However, mice engineered to express a BCL6 mutant that cannot bind to SMRT, NCOR and BCOR only manifest the GC B-cell portion of the BCL6-null phenotype [43]. T_{FH} function was entirely normal in these animals, as were Th2, Th17 and macrophage functions. Accordingly, BCL6 BTB domain-mutant mice lived normal, healthy lives and displayed no evidence of the lethal inflammatory syndrome that is characteristic of BCL6 null mice [43]. Instead, the function of BCL6 in macrophages was apparently linked to competition for DNA binding sites with STAT transcription factors. The molecular function of BCL6 in T-cells remains unknown and must be mediated through distinct and possibly novel biochemical mechanisms. For example, BCL6 regulation of the T_{FH} transcriptional program was recently shown to require cooperation with Maf [44]. In differentiating Th1 cells, BCL6 is recruited by T-bet to repress specific sets of target genes [45]. Altogether, these data indicate that BCL6 regulates cells of the immune system at least in part through distinct and specific biochemical mechanisms.

Mechanisms of BCL6 regulation in normal lymphocytes

Transcriptional and post-transcriptional mechanisms are both critically important for regulation of *BCL6* mRNA expression and protein stability in normal lymphocytes [4]. Induction of *BCL6* expression during B-cell activation is mediated through IRF8, the only factor that has so far been shown to bind and directly induce expression of *BCL6* in B-cells entering and transiting the GC reaction [46] (Figure 2a). Signaling through p110 δ phosphatidylinositolide 3-kinase (PI3K) was shown to enhance *BCL6* expression during class switch recombination, potentially through counter-regulation of NF- κ B and/or IRF4 levels [47]. In the GC, proliferating B-cells require IL-21 receptor (IL-21R) signaling to directly up-regulate and maintain expression of *BCL6* [48]. *In vivo* regulation of *BCL6* expression in GC T_{FH} cells appears to be independent of IL-21 and IL-6 [48,49], and absence of both factors, while modestly compromising early GC T_{FH} development and expansion, does not affect *BCL6* mRNA levels in these cells [50]. Instead, inducible costimulator (ICOS) was shown to be an important initiator of *BCL6* expression during early T_{FH} cell differentiation [24]. *In vitro* induction of *BCL6* expression in polarized Th1 cells appears to require the direct binding of the Foxo1 and Foxo3a transcription factors, which direct Th1 cells toward

the T_{FH} lineage [10]. What drives *BCL6* expression in B and T_{FH} cells that reside in the inter- and outer-follicular regions and participate in pre-GC B:T cell interactions is not currently known, but is proposed to involve strength of antigen stimulation, signaling through toll-like receptors, (potentially via IRF8), IL-21 or SLAM/SAP in B-cells, and in T_{FH} cells, signaling via the T-cell receptor (TCR) and costimulatory molecules, CD28 and ICOS [22].

Down-regulation of *BCL6* in exiting GC B-cells is mediated by the transcription factors IRF4 and PRDM1 [2,4], and by *miR-30* [51] (Figure 2a). In T_{FH} cells, *BCL6* expression is also down-regulated by PRDM1 [8,52]; however, IRF-4, which is required for T_{FH} cell differentiation, appears to promote *BCL6* expression in CD4+ T cells [53]. Type 1 interferons (IFN- α , - β) directly down-regulate *BCL6* mRNA levels in GC B-cells [54], likely through STAT5, which is a negative regulator of *BCL6* expression in B and T_{FH} cells [55–57]. Interferon signaling also negatively regulates *BCL6* via T-bet, to direct Th1 cell commitment [9,10]. Similarly, IL-2 appears to play a role in the regulation of *BCL6* and PRDM1 levels in T helper cells [10], repressing early commitment to the T_{FH} lineage by reducing *BCL6* expression [58]. Finally, *BCL6* can regulate its own expression in B-cells, which is also thought to occur in GC T_{FH} cells [6].

Post-transcriptional mechanisms that regulate *BCL6* protein levels, include functional inactivation of *BCL6* via p300-mediated acetylation [59] and degradation through the ubiquitin-proteasome pathway by ATM- and MAPK-mediated phosphorylation [60,61] (Figure 2b). Degradation of *BCL6* in B-cells is mediated through FBXO11-CUL1 E3 ligase complexes and the COPS5 protein of the COP9 signalosome [62,63]. In contrast, *BCL6* is protected from degradation by Hsp90, which extends the half-life both of *BCL6* protein and its mRNA [64]. At *BCL6* target genes, *BCL6* corepressor complexes are disrupted through CD40 signaling to SMRT and NCoR [65].

Dysregulation of *BCL6* in B and T cell lymphomas

BCL6 plays a fundamental role in maintaining the proliferation and survival of B-cell lymphomas that arise from GCs, as documented most clearly in the case of DLBCLs [64,66] (Figure 1a). This function arises from its role as a cell cycle and DNA damage checkpoint suppressor in GC B-cells. Indeed, the ability of *BCL6* to impair DNA damage sensing [65] may explain in part the heavy burden of mutations observed in recent genomic re-sequencing studies of DLBCL [67–69]. The oncogenic function of *BCL6* is counterbalanced in normal GC B-cells by its repression of oncogenes such as *BCL2*, *MYC* and *BM11* [41,70]; however, *BCL6* loses control of these targets in B-cell lymphomas at least in part through genetic lesions of these loci. Although *BCL6* is expressed almost universally in DLBCLs, its constitutive expression is often dependent on translocations in activated B-cell (ABC)-type DLBCLs, which originate from B-cells that would normally be down-regulating *BCL6* [71]. Somatic mutations of genes encoding regulators of *BCL6* may also enhance or maintain its functions in lymphomas. For example, somatic loss of function mutations of EP300 that disable its lysine acetyl-transferase activity may stabilize *BCL6* by maintaining Hsp90-*BCL6* complexes, as well as reducing acetylation of *BCL6* [72,73]. *BCL6* stability may also

be maintained by an inactivating mutation of FBXO11, which reduces the rate of ubiquitin-mediated BCL6 degradation [62].

The discovery of a role for BCL6 in GC T_{FH} cells and other T-cell subsets has raised the question of whether dysregulation of *BCL6* and BCL6-mediated pathways could help to drive lymphomas derived from T-cells. Indeed, BCL6 over-expression was previously detected in aberrant CD30+ T-cells that constitute ALK+ anaplastic large cell lymphomas (ALCLs) [74]. Angioimmunoblastic T cell lymphomas (AITLs) appear to express T_{FH} cell-type genes, including *BCL6* [75,76]. Cutaneous T-cell lymphomas (CTCLs) also express BCL6 and a T_{FH}-like profile, including PD-1 [77]. These studies suggest that T_{FH} cells could become malignant, or alternatively, that transformed T-cells acquire a T_{FH}-like phenotype and might be addicted to BCL6. It is possible that BCL6+ T cell subsets could support the tumor microenvironment of transformed B or T-cells, as has been shown for T_{reg} cells present in AITL and extranodal NKT-cell lymphomas [78]. Malignant cells that express FOXP3 and have similar functions to T_{reg} cells have also been identified in CTCLs and adult T cell leukemia/lymphomas [78]; however, it is not known whether BCL6 is expressed or genetically altered in these cells, nor how these cells might contribute directly to the disease. Further research is needed to understand whether T-cell lymphomas are addicted to BCL6. Peptidomimetic and small molecule inhibitors that disrupt binding of the SMRT, NCoR and BCoR corepressors to the BCL6 BTB domain potentially kill B-cell lymphoma cells [66,79]. However, given that BCL6 does not function in T-cells through this mechanism [43], it seems unlikely that these inhibitors would disrupt putative BCL6 functions in T-cell lymphomas.

Conclusions

The discovery of novel roles for BCL6 in the development and effector functions of a wide variety of mature T-cell subsets, suggest that BCL6, in addition to being a true “master regulator” of the GC reaction, is also an important regulator of T-cell-dependent inflammatory, autoimmune and memory responses in the periphery. Quite remarkably, BCL6 controls the GC reaction by mediating its effects on multiple different cell types, via distinct biochemical mechanisms of action. Advances in cell imaging have also uncovered a critical and early role for *BCL6* expression in the events leading up to GC formation. In addition to controlling the effector functions of mature lymphoid cells, BCL6 also regulates the survival of early developing pre-B-cells. Altogether, these breakthrough studies have implicated BCL6 as a central component of the wider adaptive immune system.

Precisely how BCL6 mediates its repressive effects on the gene expression programs of specialized subtypes of T helper and T regulatory cells remains to be determined, but, based on the functional effects of a mutant form of BCL6 in mice, is likely to involve distinct and potentially novel combinations of co-repressor complexes and co-factors that are T cell lineage-specific. Of critical importance to the advancement of new therapies for BCL6+ T-cell lymphomas, is an understanding of the molecular regulatory mechanisms that underlie *BCL6* expression in T cells. For example, it is not known whether blockade of the pathways that regulate BCL6 stability in B cells that successfully kill DLBCLs could also be utilized to treat BCL6-dependent T-cell lymphomas. However, it is plausible that BCL6 could

simply be a “bystander” differentiation marker of T_{FH} or T_{reg}-like T-cell lymphomas. A direct link between BCL6 and the development, maintenance and survival of T-cell lymphomas requires further investigation.

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Highlights

- BCL6 is important for pre-GC events and for T_{FH}, T_{FR} and NKT_{FH} function in GCs.
- BCL6 is a regulator of T-cell-dependent inflammation and autoimmune responses.
- BCL6 is likely to regulate B and T-cells via cell-specific biochemical mechanisms.
- Dysregulation of *BCL6* could contribute to BCL6+ T-cell lymphomas.

Figure 1.

Lymphocyte subsets that require *BCL6* for their development and effector functions. (a) GC-based lymphocyte subsets. Outside of the follicle, naïve B cells that become activated by antigen migrate to the interfollicular zone, where they up-regulate *BCL6* and participate in interactions with pre-GC T_{FH} cells, which also express *BCL6*. *BCL6*⁺ pre-GC T_{FH} cells also promote the extrafollicular antibody response and production of short-lived plasma cells. Formation of the GC reaction requires further up-regulation of *BCL6* expression in activated B-cells and involves the rapid clonal expansion and somatic hypermutation of GC B cells within the intrafollicular zone. Within the GC, high-affinity antigen-specific B-cells are provided help by *BCL6*⁺ GC T_{FH} cells and NKT_{FH} cells (and follicular dendritic cells (FDC) which do not express *BCL6*), of which GC T_{FH} cells can go on to become *BCL6*⁺ T_{FH} -like central memory T cells. The magnitude of the GC reaction appears to be regulated by *BCL6*⁺ GC T_{FR} cells. High *BCL6* expression in GC B-cells can lead to the development of GC-derived B-cell lymphomas, including GC B-cell (GCB)-type DLBCL, FL and Burkitt's Lymphoma (BL). It is not yet known whether T-cell lymphomas can also arise from this compartment. (b) Lymphoid subsets that express *BCL6* and reside outside of GCs include pre-B cells, $CXCR5^{lo}ICOS^{lo}$ T_{FH} cells, which provide help to naïve and memory B cells, Th17, T_{reg} and memory CD4⁺ and CD8⁺ T-cells. *BCL6* expression in CD4⁺ T_{reg} cells helps to regulate T-cell-dependent inflammatory and autoimmune responses. Solid arrows indicate cell differentiation pathways; dotted lines indicate cell interactions and effector functions.

Figure 2.

Molecular mechanisms of *BCL6* regulation in B and T lymphocytes. (a) Transcriptional regulators of *BCL6* induction (green arrows) and down-regulation (red bars) in B and T lymphocytes. Transcription factors, of which IRF4, IRF8, STAT5, T-bet and Foxo1 and Foxo3a bind directly to the *BCL6* gene control region, are indicated by colored shapes. Question marks signify unknown mediators and putative effects. (b) Post-translational regulation of *BCL6* protein stabilization (green arrows) and degradation (red bars) in B and T lymphocytes. Acetylation (Ac) of *BCL6* by P300 mediates *BCL6* protein degradation. Phosphorylation (P) of *BCL6* by MAPK or ATM results in *BCL6* ubiquitylation (Ub) and proteasome-mediated degradation. FBXO11 and COP5 also mediate ubiquitin-dependent degradation of *BCL6*. The HSP90 chaperone stabilizes the *BCL6* protein half-life. Question marks signify unknown mediators of *BCL6* protein stabilization and degradation in T-cells.