Inhibition of Th2 Differentiation and GATA-3 Expression by BCL-6

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The B cell lymphoma (BCL)-6 transcriptional repressor protein is an important regulator of Th2 responses. Mice deficient in BCL-6 develop severe Th2-type inflammation that can develop even in the absence of IL-4 signaling. We have investigated the mechanism for how BCL-6 regulates Th2 cell differentiation and have found that IL-6 signaling can promote dramatically increased levels of Th2 differentiation in BCL-6−/− CD4 T cells compared with wild-type CD4 T cells. IL-6 can induce a low level of Th2 cytokine expression in BCL-6−/−/STAT6−/− cells but not in STAT6−/− cells. Since the promoters for Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13 do not contain consensus BCL-6 DNA binding sites, we investigated whether BCL-6 might regulate the GATA-3 transcription factor that activates the expression of multiple Th2 cytokines. Consistent with the idea that BCL-6 represses GATA-3 expression, we found that GATA-3 levels are up-regulated in BCL-6−/−/STAT6−/− CD4 T cells compared with STAT6−/− CD4 T cells. Retrovirus-mediated expression of BCL-6 in BCL-6−/−/STAT6−/− T cells as well as developing wild-type Th2 cells leads to a potent repression of IL-4 and IL-10 secretion. Retrovirus-mediated expression of BCL-6 in both BCL-6−/−−/−STAT6−/−− and wild-type T cells also leads to a significant decrease in GATA-3 protein levels. Surprisingly, BCL-6 does not appear to regulate GATA-3 mRNA levels and thus BCL-6 appears to regulate GATA-3 expression at a posttranscriptional level. Regulation of GATA-3 protein levels is a likely key mechanism for how BCL-6 regulates Th2 cytokine expression and Th2 differentiation independently of STAT6. These data also point to a novel regulatory mechanism for BCL-6 separate from transcriptional repression.

The molecular control of differentiation of naive CD4 T cells into Th1 and Th2 cells has been the subject of intensive study over the last several years (reviewed in Refs. 1 and 2). The cytokine Th4, which is produced by many different cell types, including Th2 cells themselves, can promote Th2 differentiation by activating the STAT6 transcription factor (1, 2). An important outcome of STAT6 activation by IL-4 appears to be the up-regulation of the GATA-3 transcription factor, since GATA-3 can activate transcription of the Th2 cytokine genes IL-4, IL-5, and IL-13 (1, 2). Although other transcription factors such as c-maf, AP-1, and NFAT have been shown to be important for the control of Th2 cytokine expression, STAT6 appears to be critical for complete differentiation into Th2 cells (1, 2). Thus, STAT6-deficient (STAT6−/−) mice have greatly diminished Th2 responses (1, 2). Nonetheless, Th2 cytokines can be produced independent of the IL-4/STAT6 pathway under certain circumstances (3–5), and this pathway appears to rely on STAT6-independent up-regulation of GATA-3 (6). Recently, the transcriptional repressor B cell lymphoma (BCL)3−/− mice were found to be an important inhibitor of Th2 responses (5, 7, 8). Mice deficient in BCL-6 (BCL-6−/− mice) develop greatly enhanced Th2 cytokine responses and frequently die of Th2-type inflammation of the heart and lungs. Notably, BCL-6 regulates Th2 cytokine expression independently of STAT6, since mice deficient in both STAT6 and BCL-6 (DKO mice) were found to have Th2 cytokine responses and Th2-type inflammation similar to BCL-6−/− mice in vivo (5).

The BCL-6 gene was initially identified as a frequent target of chromosomal translocations in diffuse large cell lymphoma, a common subtype of non-Hodgkin’s lymphoma (reviewed in Refs. 9 and 10). BCL-6 encodes a 706-aa transcriptional repressor protein containing an N-terminal POZ/BTB domain and 6 zinc fingers (9, 10). BCL-6 represses transcription by recruiting corepressor proteins such as SMRT, N-CoR, and BCoR that act at least in part by recruiting histone deacetylases (11). BCL-6 is capable of binding a DNA sequence (TTT[CGT][AT]NGAA) very similar to the STAT factor DNA binding site (TTCCNNNGAA) (7, 12, 13), although whether these factors compete for binding in vivo is not clear. BCL-6 is expressed in both B and T lymphocytes and is expressed at the highest levels in B and T cells of the germinal center reaction (9, 10).

A number of target genes repressed by BCL-6 in B cells were recently identified using cDNA microarray technology; these include B cell differentiation factors (Blimp1), cell cycle regulators (cyclin D2, p27(kip1)), lymphocyte activation genes (CD44, CD69), and chemokines (macrophage-inflammatory protein 1α, IFN-inducible protein 10) (14). Chemokine genes (monocyte chemoattractant protein 1, monocyte chemoattractant protein 3, and macrophage-inflammatory protein 1α-related protein 1(C10)) are also important targets of transcriptional repression by BCL-6 in murine macrophages (15). Although overexpression of proinflammatory chemokines by macrophages provides at least a partial explanation for the inflammation in BCL-6−/− mice, this finding does not explain the abnormal Th2 responses observed in DKO mice. Additionally, there is a general lack of knowledge about target genes that are regulated by BCL-6 in T cells. In this study, we analyzed BCL-6−/− T cells to understand...
some of the molecular signals by which BCL-6 controls Th2 cytokine expression. We present evidence here that BCL-6 can affect GATA-3 expression in T cells. Increased GATA-3 levels in BCL-6-deficient T cells provides a mechanism for the STAT6-independent regulation of Th2 responses by BCL-6.

Materials and Methods

Mice

Animals were maintained under specific pathogen-free conditions in animal facilities certified by the American Association of Laboratory Animal Care. Wild-type (WT), BCL-6−/−, STAT6−/−, and BCL-6−/−/STAT6−/− (DKO) mice were maintained on a mixed C57BL/6-129 background and were genotyped by PCR as previously described (5).

T cell isolation and culture

Naïve (CD62 ligand-positive) lymph node CD4 T cells from WT, BCL-6−/−, STAT6−/−, and DKO mice were isolated by FACS as described elsewhere (5). Sorted naïve CD4 T cells were stimulated with either PMA (10 ng/ml) (Sigma-Aldrich, St. Louis, MO) plus ionomycin (0.3 μM, P+I; Sigma-Aldrich) or plate-bound anti-CD3 Ab (10 μg/ml) plus anti-CD28 Ab (2 μg/ml (Fig. 1) or 20 μg/ml (Figs. 2–4). Anti-CD3, anti-CD28, and anti-IL-4 Abs were obtained from BD Pharmingen (Santa Clara, CA). Recombinant mouse IL-6 (BD Pharmingen) was added at the start of the cultures at 10 ng/ml. Cultures were expanded with human rIL-2 (200 U/ml; Biological Resources Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD) for 3–5 days. T cells were then washed with PBS and restimulated with anti-CD3 Ab (10 μg/ml) alone. Supernatants were harvested after 24 h. IL-4 and IL-5 levels were measured by ELISA using reagents obtained from BD Pharmingen. For RT-PCR protection and RT-PCR analyses, one million sorted naïve CD4 T cells were activated with either P+I or anti-CD3 plus high anti-CD28 plus IL-6. Total RNA was prepared from the cells after 48 h with TRIZol reagent (Invitrogen, Carlsbad, CA). In some experiments, total lymph node cells were stimulated with Con A plus IL-6 for 48 h before harvesting RNA. RNase protection assays were performed as described previously (7).

RT-PCR and Western blots

RNA prepared from TRIZol lysates as above was used for cDNA preparation using the SuperScript First-Strand cDNA Synthesis System (Invitrogen). Empirically determined concentrations of first-strand cDNA were used in RT-PCR to ensure linear amplification of sequences. Primers were designed when possible (for GATA-3 and STAT6) to span intronic regions to distinguish amplification from cDNA sequences from genomic sequences. Primers for GATA-3 were 5′-CTGTTGGCTGTTACTACA GCTCTCA-3′ and 5′-ACCATGGCGGTTGCACTGGC-3′. Primers for c-Maf were 5′-ACCTGAAACCGCAGTTCGGCCGG-3′ and 5′-CT TCTCGATTTTCCTTGAGGGTC-3′. Primers for β tubulin were 5′-CACGCCCCGAGATGGTGCACAG-3′ and 5′-GGCTCTATTAGTACACAGAGATT-3′. Whole cell lysates and immunoblots were prepared with standard techniques. Blots were probed with anti-GATA-3 mAb (clone HG3-31; Santa Cruz Biotechnology, Santa Cruz, CA). GATA-3 blots were developed with ECL reagents (Amersham Pharmacia Biotech, Piscataway, NJ). After anti-GATA-3 probing, blots were probed with Ab to β-actin (Sigma-Aldrich) to demonstrate equivalent protein loading.

Retroviral infection of T cells

The pMIEG3 and pMIEG3-BCL-6 plasmids were described previously (15). Phoenix-eotropic retroviral producer cells were grown and transduced when possible (for GATA-3 and STAT6) or plated-bound anti-CD3 as above. IL-4 and IL-10 levels were measured by Quantikine ELISA kits (BD Pharmingen). IFN-γ was measured with an immunoassay kit obtained from R&D Systems (Minneapolis, MN).

Results

T cell defects in BCL-6−/− mice

Previous data indicated that BCL-6−/− T cells were required but were not sufficient to drive the Th2-type inflammation (15). Since T cells are required for the inflammation in BCL-6−/− mice, BCL-6−/− T cells may act either as passive intermediary cells in the development of inflammatory disease or may have a unique defect that specifically augments Th2-type inflammation. Previously, we found that naive CD4 T cells from BCL-6−/− mice can be programmed into either Th1 or Th2 cells by normal signals, i.e., either IL-12 or IL-4, respectively (5). Furthermore, we found that CD4 T cells from BCL-6−/−/STAT6−/− double mutant mice (DKO mice) did not develop into Th2 cells in response to IL-4 signaling, showing that under these in vitro conditions, BCL-6−/− T cells do not undergo abnormal Th2 differentiation (5). We therefore wondered whether the abnormal Th2 differentiation of BCL-6−/− T cells in vivo might be explained by the actions of other cytokines besides IL-4 and IL-12. We decided to test whether IL-4, a T cell costimulatory factor that has been shown to promote Th2 differentiation (17, 18), could promote the Th2 differentiation of BCL-6−/− T cells. To explore this idea, we undertook a more extensive analysis of T cells from BCL-6−/− mice. For these experiments, we used naïve (CD62 ligand-positive) CD4 T cells isolated by FACS for in vitro differentiation assays, where IL-4 production after secondary stimulation is a measurement of Th2 differentiation. As shown in Fig. 1, BCL-6−/− T cells activated with anti-CD3 plus anti-CD28 Abs alone did not undergo significant Th2 differentiation, indicating that our preparations of BCL-6−/− CD4 T cells did not contain significant numbers of preexisting Th2 cells. These data also support the idea that BCL-6−/− T cells are not intrinsically biased to becoming Th2 cells. Strikingly, we found that IL-4 plus anti-CD3 plus anti-CD28 was a very potent promoter of Th2 differentiation of BCL-6−/− but not WT naive CD4 T cells (Fig. 1). We also tested whether IL-6 could induce Th2 differentiation in STAT6−/− and DKO (BCL-6−/−/STAT6−/−) T cells. T cell activation in the

![FIGURE 1. Increased Th2 differentiation after IL-6 costimulation of BCL-6−/− T cells. Cytokine production after in vitro T cell differentiation cultures under conditions noted using naive CD4 T cells from WT, BCL-6−/−, STAT6-deficient (STAT6−/−), and BCL-6−/−/STAT6-deficient (DKO) mice. * Not detectable. The data shown are representative of three separate experiments, each using different preparations of sorted cells.](attachment:image)
presence of IL-6 led to a low but substantial amount of IL-4 production from DKO T cells and no detectable IL-4 from STAT6−/− T cells (Fig. 1). Although clearly much of the IL-4 production from BCL-6−/− T cells is IL-4-STAT6 dependent, it is nonetheless remarkable that IL-6 can induce some degree of Th2 differentiation of DKO T cells in vitro. Moreover, the IL-4 produced by IL-6-stimulated DKO T cells was greater than that produced from IL-6-stimulated WT T cells. Thus, IL-6 can promote the development of IL-4-secreting cells from DKO T cells in vitro. IL-6-differentiated DKO cells also produce significant levels of the Th2 cytokines IL-5, IL-10, and IL-13 (Fig. 2 and data not shown), and thus IL-6 stimulation in the absence of BCL-6 reveals a novel STAT6-independent pathway for Th2 cytokine production. This pathway may account for the Th2 cytokines and Th2-type inflammation found in DKO mice (5).

**Regulation of cytokine gene expression by BCL-6**

To analyze in more detail how BCL-6 was affecting cytokine expression, we used an RNase protection assay to examine the same preparations of naive CD4 T cells as used in Fig. 1. At 48 h after activation with P+1 alone, both WT and BCL-6−/− T cells showed that IL-2 was the dominant cytokine mRNA expressed and neither cell type showed detectable expression of mRNAs for Th2 cytokines such as IL-4, IL-5, IL-10, or IL-13 (Fig. 2). This finding was consistent with our results in differentiation cultures (Fig. 1a), where P+1 did not promote Th2 differentiation of either WT or BCL-6−/− cells. In contrast, 48 h after naive CD4 T cell activation with strong costimulation (anti-CD3 and anti-CD28 Abs plus IL-6), there were significant differences between WT and BCL-6−/− T cells (Fig. 2). With strong costimulation, WT and STAT6−/− T cells showed a similar cytokine expression pattern as with P+1 stimulation. In contrast, with strong costimulation, BCL-6−/− and DKO T cells both displayed a significant up-regulation of the Th2 cytokine genes IL-4, IL-10, and IL-13 compared with WT and STAT6−/− cells, respectively. In particular, BCL-6−/− T cells activated with strong costimulation showed a large up-regulation of expression of mRNAs for the Th2 cytokines IL-4, IL-10, and IL-13. Consistent with the differentiation cultures in Fig. 1, treatment of BCL-6−/− cells with anti-IL-4 Abs blocked much of the up-regulation of the Th2 cytokines (Fig. 2). DKO cells showed a similar pattern to BCL-6−/− cells treated with anti-IL-4 Abs. Both DKO and BCL-6−/− cells showed a large up-regulation of IL-10 gene expression compared with WT and STAT6−/− controls (Fig. 2, lanes 4, 6, 8, and 10). Most significantly, the hallmark Th2 cytokines IL-4, IL-5, and IL-13 were up-regulated in DKO T cells compared with STAT6−/− T cells (Fig. 2, lanes 13 and 14). Since STAT6 deficiency blocks normal Th2 differentiation, the increase in Th2 cytokine mRNAs most likely reflects changes in gene expression due to lack of BCL-6. Thus, the Th2 cytokines IL-4, IL-5, IL-10, and IL-13 are specifically regulated by BCL-6 during T cell activation.

**Up-regulation of GATA-3 in the absence of BCL-6**

BCL-6 might affect the expression of Th2 cytokine genes by binding to regulatory sequences in each of these cytokine genes to repress the transcription of these genes. Alternatively, BCL-6 might affect the expression of Th2 cytokine genes by inhibiting a transcription factor that positively regulates Th2 differentiation and cytokine transcription. Either possibility would lead to increased transcription of these genes in BCL-6−/− T cells. Using the consensus DNA binding site for BCL-6 (12), we examined the promoters for the mouse IL-4, IL-5, IL-10, and IL-13 genes for possible BCL-6 binding sites and found that none of these promoter sequences contained a consensus BCL-6 binding site. We therefore pursued the hypothesis that BCL-6 might regulate the expression of Th2 cytokines indirectly, by inhibiting the expression of another transcription factor. An obvious candidate for such a target is the GATA-3 transcription factor that is a well-characterized activator of Th2 cytokine expression (reviewed in Refs. 1 and 2). We initially investigated whether the burst of Th2 cytokine expression seen in BCL-6−/− T cells 48 h after strong costimulation (Fig. 2) correlated with an increase in GATA-3 expression. We stimulated naive CD4 T cells from WT and BCL-6−/− mice for 48 h with anti-CD3 plus anti-CD28 plus IL-6 as in Fig. 2 and then prepared RNA after 48 h. RT-PCR analysis demonstrated a large increase in GATA-3 gene expression in BCL-6−/− T cells but not WT T cells under these activation conditions (Fig. 3a). Addition of anti-IL-4 Ab to the BCL-6−/− culture blocked the up-regulation of GATA-3 (Fig. 3a), indicating that much of the GATA-3 up-regulation was due to feedback induction of GATA-3 by IL-4 produced from the BCL-6−/− T cells. The same pattern of GATA-3 expression was observed when GATA-3 protein was analyzed (Fig. 3b).

Consistent with the known role of GATA-3 in inducing Th2 cytokine expression, the increased levels of GATA-3 in BCL-6−/− T cells stimulated with IL-6 correlate with the pattern of Th2 cytokine expression observed in these cells in Figs. 1 and 2. Three major possibilities can explain the large increase in GATA-3 levels in IL-6-stimulated BCL-6−/− cells. First, BCL-6 may directly regulate IL-4 and thus an increased secretion of IL-4 in BCL-6−/− T cells following IL-6 stimulation could lead to greater GATA-3 up-regulation. We think this is relatively unlikely because we have not been able to detect a large burst of IL-4 transcription after costimulation of BCL-6−/− T cells (data not shown) and because we did not identify BCL-6 binding sites in the IL-4 promoter or in the known IL-4 regulatory regions. A second possibility is that BCL-6−/− cells secrete normal amounts of IL-4 after activation but STAT6 signaling is augmented, leading to increased induction of GATA-3 by the IL-4–STAT6 pathway. We also think this possibility is unlikely because we do not observe up-regulation of

![FIGURE 2](image-url). RNase protection analyses of cytokine mRNAs. Naive CD4 T cells from WT, BCL-6−/−, STAT6−/−, and DKO mice were stimulated for 48 h with either P+1 (lanes 1 and 2) or anti-CD3 plus anti-CD28 Abs plus IL-6 (lanes 3–14). Cells used in lanes 4, 6, 8, and 10 also were treated with anti-IL-4 Ab. Lanes 11–14 are a separate experiment from lanes 7–10 and lanes 13–14 represent a longer exposure time for the same assay as in lanes 11 and 12. The data shown are representative of two separate assays.
other STAT6-responsive genes (such as the IL-4Rα chain) in BCL-6−/− cells after activation in the presence of IL-4 (data not shown). The third possibility is that GATA-3 is directly regulated by BCL-6 and GATA-3 levels are thus increased in BCL-6−/− T cells. In this scenario, increased GATA-3 levels after activation with IL-6 lead to an initial increase in IL-4 transcription, which leads to feedback amplification of the IL-4-STAT6-GATA-3 pathway.

In addition to GATA-3, another important transcription factor for Th2 cytokine expression is c-Maf (1, 2). We did not detect significant changes in c-Maf expression in BCL-6−/− vs WT T cells after IL-6 stimulation (Fig. 3a), making it unlikely that BCL-6 modulates Th2 differentiation by regulating c-Maf levels.

We therefore analyzed further the regulation of GATA-3 expression by BCL-6. To remove the effects of IL-4-STAT6 signaling on GATA-3 expression, we examined GATA-3 expression in STAT6-deficient (STAT6−/− and DKO) lymph node T cells stimulated with Con A with or without IL-6 for 48 h. To carefully quantitate GATA-3 mRNA levels by RT-PCR, we titrated the amount of input cDNA (Fig. 4a). As a control, we amplified the housekeeping gene β tubulin and observed similar linear levels of amplification between STAT6−/− and DKO cells. Titration of the cDNA also showed linear levels of amplification for GATA-3. Essentially similar levels of GATA-3 message were detected in DKO cells and STAT6−/− cell at all levels of input, although very slightly higher levels of GATA-3 mRNA were observed in DKO cells compared with STAT6−/− cells. To assess GATA-3 expression more completely, we analyzed GATA-3 protein levels by Western blot (Fig. 4b). Similar levels of GATA-3 were expressed by both STAT6−/− and DKO T cells activated with Con A alone. Surprisingly, IL-6 stimulation led to a decrease in GATA-3 protein expression in both STAT6−/− and DKO T cells. However, whereas IL-6 led to an almost complete loss of GATA-3 expression in STAT6−/− T cells, DKO T cells still expressed significant levels of GATA-3 protein (Fig. 4b). These data therefore indicate that both BCL-6 and IL-6 can control levels of GATA-3 protein in the absence of IL-4-STAT6 signaling. Moreover, BCL-6 and IL-6 appear to control GATA-3 levels by a posttranscriptional mechanism.

**Inhibition of GATA-3 expression and Th2 cytokine expression in DKO T cells by BCL-6**

We next wanted to confirm that BCL-6 could regulate GATA-3 expression directly in T cells. We therefore used a retroviral expression system to introduce BCL-6 into primary T cells. BCL-6 is expressed in resting T cells, but is normally down-regulated after T cell activation (19). Expression of BCL-6 in WT T cells by retrovirus serves as a way of maintaining BCL-6 expression during T cell differentiation. Additionally, expression of BCL-6 in DKO T cells serves as a way of correcting the genetic deletion of BCL-6 in these T cells. We therefore infected DKO T cells with Con A plus IL-6 and infected them with control and BCL-6 retroviruses to test whether BCL-6 could inhibit the increased GATA-3 expression and increased Th2 cytokine expression in these BCL-6-deficient cells. Both control and BCL-6 retroviruses express GFP and infected T cells were isolated by GFP expression. Sorted cells were assessed for levels of GATA-3 protein and β tubulin protein by Western blot. The data shown are representative of two different assays.
infected T cells were isolated on the basis of GFP expression. Immediately after sorting, we prepared whole cell lysates from the infected cells and analyzed GATA-3 protein levels by Western blot. We observed a significant down-regulation in GATA-3 protein in the BCL-6-expressing cells (Fig. 5). We next tested whether BCL-6 retroviruses could also affect IL-4 and IL-10 cytokine secretion by the DKO cells (Fig. 6). Expression of BCL-6 in the DKO T cells led to >90% inhibition of both IL-4 and IL-10 expression. Thus, BCL-6 represses expression of GATA-3 and Th2 cytokines in DKO T cells, indicating that BCL-6 is regulating Th2 differentiation independent of IL-4 signaling and also in a T cell autonomous manner.

**Inhibition of GATA-3 expression and Th2 cytokine expression in WT T cells by BCL-6**

We next tested whether BCL-6 might inhibit the Th2 differentiation of WT T cells. We stimulated WT T cells with Con A plus IL-4 and infected them with control and BCL-6 retroviruses and isolated infected cells via GFP expression. After sorting, the cells were split into two groups and either lysed for Western blot analysis or restimulated with anti-CD3 Ab for analysis of cytokine secretion. Western blot analysis showed that GATA-3 levels in the WT Th2 cells were significantly reduced by expression of BCL-6 (Fig. 7). Cytokine secretion from the control and BCL-6-infected T cells was measured by ELISA (Fig. 8). Expression of BCL-6 in CD4 T cells led to a strong inhibition of IL-4 secretion and almost complete inhibition of IL-10 expression. In contrast, BCL-6 only weakly affected the ability of these cells to produce IFN-γ. Thus, BCL-6 is able to specifically repress the expression of Th2 cytokines in T cells. These data confirm that BCL-6 is a T cell autonomous regulator of Th2 differentiation.

**Posttranscriptional regulation of GATA-3 by BCL-6**

Lastly, we tested whether BCL-6-regulated GATA-3 expression in T cells by down-regulation of GATA-3 mRNA or by a different mechanism. We analyzed GATA-3 mRNA levels by RT-PCR in the retrovirus-infected WT Th2 cells (Fig. 9). We did not observe...
any significant down-regulation of GATA-3 mRNA by the BCL-6 retrovirus. These data indicate that BCL-6 may affect GATA-3 expression at the posttranscriptional level and are consistent with the data in Fig. 3 that also suggest a posttranscriptional regulation of GATA-3 by BCL-6.

Discussion

In this study, we have characterized the regulation of cytokine transcription and Th2 differentiation by the transcriptional repressor protein BCL-6. We found that BCL-6 plays a particularly critical role in Th2 cytokine expression and Th2 differentiation in response to T cell costimulation with IL-6. Strikingly, Th2 cytokines can be induced in BCL-6-deficient T cells by IL-6 costimulation even in the absence of STAT6 signaling. Since these results indicated to us that BCL-6 might regulate Th2 differentiation in a T cell autonomous fashion, we definitively tested this idea by inserting BCL-6 into differentiating Th2 cells. We found that BCL-6 can potently inhibit Th2 cell differentiation in a T cell autonomous fashion. We also tested whether BCL-6 can regulate expression of the GATA-3 transcription factor that is critical for inducing Th2 cytokine expression and Th2 differentiation. We found that BCL-6 can repress GATA-3 protein levels in DKO T cells and in developing WT Th2 cells. GATA-3 is also up-regulated in DKO T cells stimulated with IL-6. Taken together, these findings indicate that GATA-3 is regulated by BCL-6 in T cells. The regulation of GATA-3 by BCL-6 is independent of STAT6. These results reveal an important mechanism for how BCL-6 can influence Th2 cytokine expression and Th2 differentiation. Specifically, the up-regulation of GATA-3 in BCL-6−/− T cells can explain the bias toward Th2-type responses in BCL-6−/− mice and even in DKO mice.

Multiple studies have implicated GATA-3 in Th2 cell commitment and function (reviewed in Refs. 1 and 2). One important function for GATA-3 is to bind to the IL-4 enhancer and other regulatory regions in the IL-4 gene with the purpose of augmenting transcription (20, 21). GATA-3 can also directly transactivate the IL-5 and IL-13 promoters (22–25). There is also some data that suggest that GATA-3 can promote IL-10 transcription (6, 26). It is not clear whether GATA-3 directly activates IL-10 or whether GATA-3 indirectly activates IL-10 transcription in Th2 cells via another transcription factor. In either case, GATA-3 is clearly a key factor in Th2 cell differentiation and Th2 cytokine expression. The regulation of GATA-3 by BCL-6 provides an important mechanism for the severe Th2-type inflammatory disease seen in both BCL-6−/− and DKO mice.

Since our data indicate that BCL-6 can modulate GATA-3 expression, an important question is how BCL-6 mechanistically affects GATA-3 expression. In this study, we present evidence that BCL-6 can repress GATA-3 protein levels in both DKO T cells stimulated in the presence of IL-6 and in WT T cells stimulated in the presence of IL-4. Strikingly, BCL-6 appears to have minimal effects on GATA-3 mRNA levels. One possibility to explain these results is that BCL-6 negatively affects GATA-3 protein levels, such as by regulating GATA-3 protein stability. Thus, in the presence of BCL-6, in WT T cells, GATA-3 protein may be less stable, and there would be relatively less GATA-3 protein without a change in mRNA levels. In the absence of BCL-6, in DKO T cells stimulated with IL-6, GATA-3 protein would be predicted to be more stable, thus increasing GATA-3 protein levels compared with WT T cells. How BCL-6 and IL-6 act together to regulate GATA-3 protein stability is not clear, although the data from Fig. 4 suggest that IL-6 decreases GATA-3 levels and that BCL-6 accentuates this effect. Thus, the effect of IL-6 is minimized in BCL-6−/− and DKO cells, resulting in a relative increase in GATA-3. IL-6 is also known to promote up-regulation of IL-4 transcription through a mechanism involving NFATc2 (18). Thus, IL-6 stimulation in BCL-6−/− T cells results in a combination of increased IL-4 expression and increased GATA-3 protein levels, and this is likely to explain both the skewed Th2 differentiation and the abnormal expression of Th2 cytokines in BCL-6−/− T cells. In terms of how BCL-6 might affect GATA-3 protein stability, very little is known about posttranscriptional control of GATA-3 expression. However, recent work has shown that GATA-3 protein is acetylated (27). Since BCL-6 can interact with histone deacetylases, BCL-6 may also interact with GATA-3 and decrease the acetylation state of GATA-3. This type of interaction could lead to increased degradation of GATA-3 protein, since acetylation can be an important factor in protein stability (28). Further work will be required to determine whether BCL-6 and GATA-3 interact and whether protein acetylation is part of the mechanism for this regulation.

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