Research Highlight

**BATF: Bringing (in) Another Th17-regulating Factor**

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T helper (Th) 17 cells are a recently identified subset of T cells that regulate tissue inflammation, and RORγt and RORα have been shown to be Th17-specific transcription factors that mediate Th17 cell generation. A new study of Batf-deficient mice shows that this AP-1 family transcription factor also regulates Th17 cell differentiation by binding to Th17-associated gene promoters and by maintaining RORα and RORγt expression, shedding new lights on current clinical modulation of Th17 cell function in inflammatory diseases.

CD4+ T helper (Th) cells play a crucial role in regulating immune responses by orchestrating the function of other immune cell types. Effector Th cell subsets are characterized by their differential cytokine production profiles and immune regulatory functions. Recently, a new Th lineage has been identified and termed Th17 cells, which produce pro-inflammatory cytokines interleukin (IL)-17, IL-17F, IL-22 and IL-21, and are regulated by unique cytokine stimuli (Dong, 2008). The differentiation of Th17 cells is mediated by RORα and RORγt, both of which are highly expressed in Th17 cells than in other types of T cells (Martinez et al., 2008; Yang et al., 2008). Moreover, Irf4 and Ahr have also been shown to regulate the expression of IL-21 and IL-22, respectively (Huber et al., 2008; Veldhoen et al., 2008). However, it is not entirely understood how Th17 differentiation is regulated at the transcriptional level, or whether other transcription factors participate in the transcriptional programming toward Th17 cells and cooperate with retinoid acid receptor-related orphan receptors (RORs) in the generation of Th17 cells. In a recent *Nature* paper, Murphy and colleagues demonstrated that the AP-1 B-cell activator transcription factor (Batf) regulates Th17 cell differentiation and cytokine production (Schraml et al., 2009). Their findings indicate that Batf regulates the expression of Th17-associated cytokines by directly binding to their promoter regions or intergenic regions and by maintaining RORγt and RORα expression (Schraml et al., 2009) (Figure 1).

Batf mRNA expression is significantly upregulated in activated Th cells and in all Th subsets, including Th1, Th2 and Th17 cells, so it is not unique to Th17 cells in terms of its expression, though no information on protein expression data was provided. To determine whether Batf regulates all these effector cells, Schraml et al. generated Batf-deficient mice. These mice did not show any defect in development of immune cells. By using T cells lacking Batf, the authors showed that Batf is not required for appropriate Th1 or Th2 cells generation *in vivo*. However, the differentiation of Th17 cells was completely impaired. Moreover, these mice also failed to produce IL-17 in both CD4+ and CD8+ T cells *in vivo*. Consistent with this finding, Batf-deficient mice were resistant to experimental autoimmune encephalomyelitis (EAE), an autoimmune disease where Th17 cells have been shown to play an indispensable pathogenic role (Martinez et al., 2008). To further demonstrate that these mice did not have an intrinsic defect in development of the disease, the authors adoptively transferred WT naïve CD4+ T cells into Batf−/− mice and demonstrated that EAE disease was completely restored under such conditions. Thus, on the basis of these results, the authors demonstrated that Batf is critically required to induce Th17 cells both *in vitro* and *in vivo* (Schraml et al., 2009).

The minimal conditions required to induce Th17 differentiation *in vitro* involve both transforming growth factor-beta (TGF-β) and IL-6 (Dong, 2008). Thus, lack of Th17 cell induction in Batf-deficient cells could be due to a defect in either cytokine signaling. To address this point, Schraml et al. demonstrated that Batf-deficient mice were able to mount normal IL-6-induced liver acute phase responses. Moreover, STAT3 phosphorylation and IL-6R upregulation by IL-6 were not affected either in Batf-deficient cells. Furthermore, genes induced by TGF-β were not altered in cells lacking Batf. Thus, the authors concluded that in Batf−/− cells, both proximal IL-6 and TGF-β signaling pathways were not disturbed. However, the authors did find a subset of IL-6-induced genes that were affected by Batf deficiency. It has been previously shown in M1 mouse myeloid leukemia cells that IL-6 in a STAT3-dependent manner induces Batf expression (Senga et al., 2002). Thus, whether induction of Batf in Th17 cells is STAT3-dependent still remains to be determined (Figure 1). Furthermore, it is plausible that IL-6-responsive genes affected in Batf-deficient cells might depend on Batf induction. Moreover, the kinetics for Batf expression in Th cells remains unknown and could also provide useful information in our understanding on the sequential
events leading to the expression of Th17-associated genes.

In an attempt to understand the molecular mechanism whereby Batf regulates Th17 cell generation, Murphy and colleagues studied the expression and role of the nuclear orphan receptors RORα and RORγt in Batf-deficient T cells. The authors found that Batf-deficient cells were capable of inducing both RORα and RORγt at an early time point. However, these cells were incapable of maintaining their expression. Thus, the exact mechanisms that lead to the induction and maintenance of RORS expression need to be fully understood. Also, whether Batf directly binds to genes encoding these transcription factors and regulates their expression needs further clarification (Figure 1).

Since RORα and RORγt expression is not maintained in Batf-deficient cells, and given that we have previously shown that both RORα and RORγt expression are required to induce full Th17 cell differentiation (Yang et al., 2008), the authors determined the effect of RORγt overexpression in Batf-deficient cells. Surprisingly, overexpression of the orphan nuclear receptor RORγt in Batf−/− cells failed to restore Th17 cell generation. Moreover, Schraml et al. also demonstrated by overexpression studies that Batf synergizes with RORγt in induction of Th17 cells. Interestingly, these two transcription factors bind to an overlapped conserved region in the sequence upstream of the IL-17 gene (Schraml et al., 2009).

Th17-associated cytokines IL-17, IL-17F, IL-21 and IL-22 have conserved binding sites for Batf in either their promoters or intergenic regions. Thus, Schraml et al. analyzed Batf binding to these promoters and found that Batf forms a heterodimer with JunB in Th17 cells, and binds to IL-17, IL-21 and IL-22 promoters as well as two intergenic regions between the IL-17A and the IL-17F genes (Figure 1). It has been previously suggested that Batf functions as a repressor of AP-1 activity. Thus, whether AP-1 inhibits the induction of Th17-associated genes needs to be further demonstrated. Nevertheless, Schraml et al. in this manuscript demonstrated that Batf, by binding to JunB, positively regulates Th17-associated genes. Given that it has been shown that IL-21 is a crucial cytokine driving Tfh differentiation (Nurieva et al., 2008; Vogelzang et al., 2008), it would be interesting also to address whether Batf is required for Thf cell induction.

This study presented convincing data supporting the role of Batf in Th17 cell generation. However, it also opens new questions that yet remain to be answered. One of these is whether binding of RORγt to IL-17 promoter is Batf-dependent. Also, do Batf and RORγt cooperate in the recruitment of chromatin remodeling complexes to allow for the opening of the chromatin structure? Is Batf by itself able to induce Th17 cells generation in the absence of RORγt? Answers to these questions will definitely provide us a better understanding of the sequential regulation of Th17-associated gene expression.

Batf is not only highly upregulated in Th17 cells but also in T cells after activation and in Th1 and Th2 cells. But, the factor(s) driving Batf expression in each Th lineage is not clear at this time. Then, why do not Th1 or Th2 cells express Th17-specific cytokines? To address this point, the authors suggest that ‘because Batf is also expressed in Th1 and Th2 cells, it probably cooperates with other Th17-specific factors to regulate target genes in Th17 cells’. To support this, the authors showed that Batf synergizes with RORγt in induction of IL-17 production, suggesting that Batf plays a non-redundant role during Th17 lineage commitment. However, overexpression of Batf by itself can lead to induction of IL-17 production. The mechanism for such upregulation remains unclear. Whether Batf synergizes with Ahr or Irf4 in induction of IL-22 and IL-21, respectively, also needs further study.

The authors also showed that Batf−/− mice had decreased regulatory T cells in steady-state conditions, although the regulatory T cell levels were comparable to WT upon immunization. Even though it was not the main point of the manuscript, this also raises the question of whether Batf is also required for induction or maintenance of regulatory T cells. However, in vitro the authors clearly demonstrated that TGF-β was capable of inducing Foxp3
expression from naïve T cells lacking Batf, generating inducible regulatory T cells. Thus, the mechanism by which Batf regulates regulatory T cell homeostasis, maintenance or generation in vivo still remains unclear.

To summarize, the work by Murphy and colleagues unravels the role of an atypical AP-1 transcription factor Batf in Th cell differentiation and provides new insight into possible cooperation between multiple transcription factors in the induction of Th-specific cytokines. Analysis of other AP-1 transcription factors and their interaction/competition with each other as well as with other transcription factors will provide a better understanding on how combinatorial signaling pathways mediate the commitment of naïve Th cells into Th-specific lineages.

References