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INSIGHTS

Regulating the regulator: Bhlhe40 directly keeps IL-10 in check

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In this issue of *JEM*, two complementary manuscripts by Huynh et al. (https://doi.org/10.1084/jem.20171704) and Yu et al. (https://doi.org/10.1084/jem.20170155) demonstrate that the transcription factor Bhlhe40 acts as a repressor of IL-10 production during infection with *Mycobacterium tuberculosis* or *Toxoplasma gondii*. Deletion of Bhlhe40 in both cases resulted in chronic infection and increased pathogen load as a consequence of increased IL-10 production.

IL-10, produced by most cells of the immune response, including macrophages, dendritic cells, and T cells, predominantly operates through the inhibition of proinflammatory cytokines to prevent immune pathologies such as colitis. In contrast, overproduction of IL-10 can lead to chronic infection (Gabrysova et al., 2014). Therefore, regulation of IL-10 is under tight control to ensure an appropriate response to eliminate pathogens is elicited, while avoiding host collateral damage. An understanding of the transcription factors regulating *Il10* gene expression and the consequences of perturbation of their function is still unclear.

The cDNA encoding Bhlhe40 (basic helix–loop–helix [bHLH] family member e40) was identified as Dec1, cAMP-dependently expressed in differentiated human embryo chondrocytes (Shen et al., 1997); Sharp2 expressed in the developing central nervous system (Rossner et al., 1997); and Stra13 (Boudjelal et al., 1997). Stra13 is a retinoic acid–inducible gene in embryonic carcinoma cells encoding a bHLH protein with high sequence identity in the bHLH domain with the *Drosophila melanogaster* Hes1 proteins, many of which are transcriptional repressors (Sun and Taneja, 2000). Stra13 was suggested to play a key role in signaling pathways that lead to growth arrest and terminal differentiation by repression of target genes via histone deacetylase–dependent as well as histone deacetylase–independent mechanisms. Dec1 was later reported to play a role in the circadian system (Honma et al., 2002).

Stra13-deficient mice developed lymphoid organ hyperplasia and autoimmunity with age (figure showing role of Bhlhe40 in steady state, autoimmunity, and infection), but did not exhibit any discernible phenotypic differences in young mice (Sun et al., 2001). *Stra13*-deficient CD4$^+$ T cells also showed defects in proliferation and IL-2, IFN-γ, and IL-4 production. The systemic autoimmune disease in aging *Stra13*-deficient mice was attributed to impaired activation-induced cell death and accumulation of activated lymphocytes. An alternative explanation was provided after the discovery of Foxp3-expressing regulatory T (T reg) cells because *Dec1*-deficient mice showed reduced Foxp3$^+$ T reg cells accompanying the lymphoproliferative disease (Miyazaki et al., 2010; figure showing role of Bhlhe40 in steady state, autoimmunity, and infection). The development of TGF-β–driven Foxp3 expressing induced T reg cells from *Dec1*-deficient naive CD4$^+$ T cells was similarly reduced, but not that of T_H17 cells in response to TGF-β/IL-6/IL-23, suggesting that TGF-β signaling was intact. Instead, Dec1 regulated and contributed to the long-term expression of the IL-2Rα through cooperative binding to the *Il2ra* gene locus with Runx1 (Miyazaki et al., 2010). Dec1 was then found to be highly induced in CD4$^+$ T cells by CD28-dependent signaling, and *Dec1*-deficient CD4$^+$ T cells showed defects in survival and proliferation and produced much reduced levels of IL-2 (Martínez-Llordella et al., 2013), in keeping with the previous findings (figure showing role of Bhlhe40 in steady state, autoimmunity, and infection). In contrast to the steady-state phenotype in vivo, Dec1 was shown to be required for the development of antigen/adjuvant-induced experimental autoimmune encephalomyelitis (EAE) and production of GM-CSF, IFN-γ and IL-2 (figure showing role of Bhlhe40 in steady state, autoimmunity, and infection). In a parallel study, mice deficient for Bhlhe40, the now official name for Dec1/Stra13, were also found to be resistant to the induction of EAE, where, in addition to demonstrating positive regulation of GM-CSF and IFN-γ, Bhlhe40 additionally negatively regulated the production of IL-10 (Lin et al., 2014). Abrogation of IL-10 signaling in *Bhlhe40*-deficient mice rendered them susceptible to EAE, identifying IL-10 as the main driver reinforcing the resistant phenotype in *Bhlhe40*-deficient mice during EAE. Whether the effects of Bhlhe40 on proinflammatory cytokines and IL-10 were direct or indirect remained to be determined.

Insight from Leona Gabryšová and Anne O'Garra.

In this issue, Yu et al. demonstrate that the Bhlhe40-mediated decrease in IFN-γ production by CD4$^+$ T cells is independent of the T_H1 master regulator transcription factor T-bet and only partially dependent on IL-10, suggesting a possible direct effect

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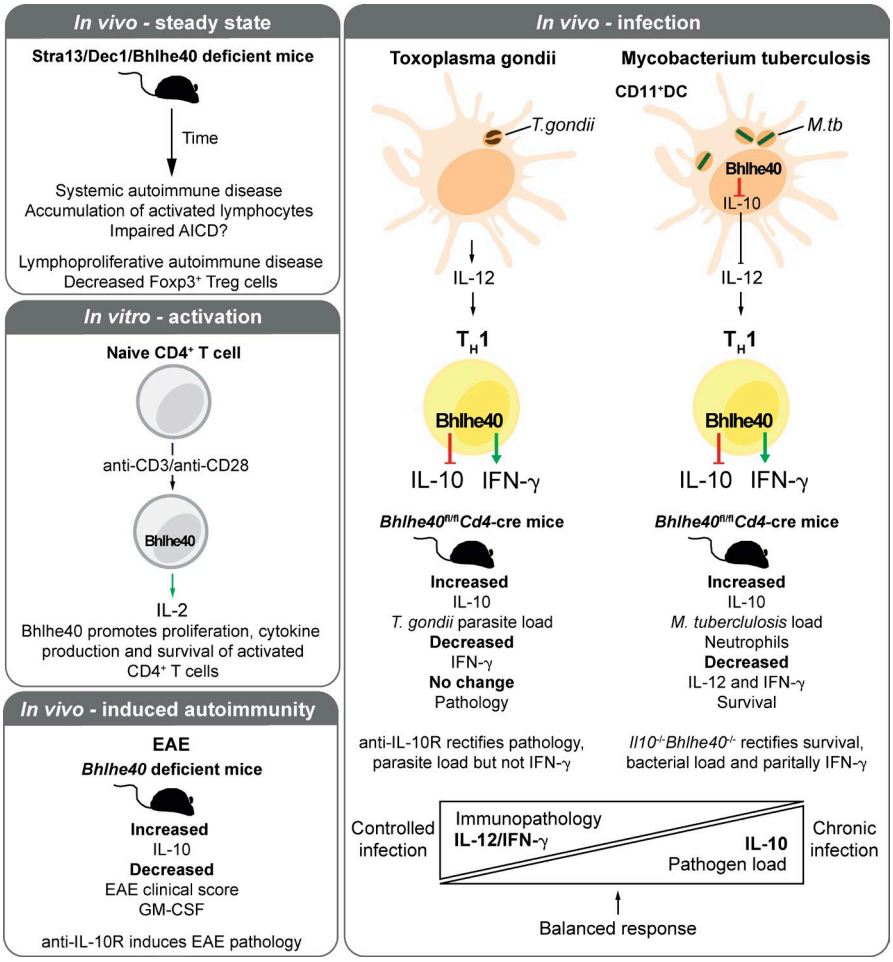
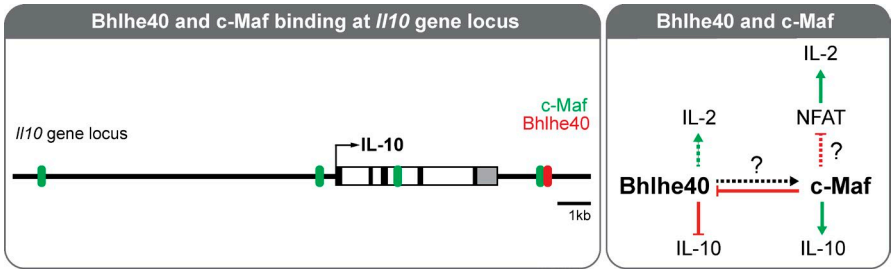


Role of Bhlhe40 in steady state, autoimmunity, and infection: Top left: Bhlhe40-deficient mice develop systemic lymphoproliferative autoimmune disease with age as a result of decreased T reg cells. Middle left: Bhlhe40 expression in naive CD4+ T cells is upregulated by anti-CD28 after activation with anti-CD3 in vitro and thus promotes proliferation, IL-2 production, and survival of CD4+ T cells. Bottom left: Bhlhe40-deficient mice are less susceptible to EAE because of increased IL-10 production. Right: Bhlhe40 deletion in T cells makes mice more susceptible to infection with *Toxoplasma gondii* and with *Mycobacterium tuberculosis*, tipping the balance between proinflammatory IL-12/IFN-γ and anti-inflammatory IL-10 production.

of Bhlhe40 on IFN-γ (figure showing role of Bhlhe40 in steady state, autoimmunity, and infection). Accompanying the decrease in IFN-γ, the production of IL-10 was substantially increased in *Bhlhe40*-deficient T_H1 cells, which was also accompanied by an increase in c-Maf expression. This suggests that Bhlhe40 may exert at least some of its repressive effects on *Il10* expression by repression of c-Maf, a major regulator of *Il10* gene expression (Gabrysova et al., 2014, 2018). Yu et al. (2018) additionally showed that mice with a conditional deletion of *Bhlhe40* in T cells succumbed to *Toxoplasma gondii* infection. This was accompanied by decreased antigen-specific IFN-γ–producing T cells, increased IL-10–producing T cells, and an increased parasite burden, with no change in the levels of a liver enzyme aspartate transaminase, suggesting their death was a result of uncontrolled infection and not immune pathology. Blockade of IL-10 signaling during infection rescued these mice from death, reversing the increased parasite load, but only partially restoring the levels of IFN-γ. In this same issue of *JEM*, Huynh et al. demonstrate that Bhlhe40 is an essential repressor of IL-10 during *Mycobacterium tuberculosis* infection (figure showing role of Bhlhe40 in steady state, autoimmunity, and infection). The loss of Bhlhe40 resulted in elevated levels of *Il10* expression, increased mycobacterial burden, and early susceptibility, strikingly to the extent observed in mice lacking IFN-γ. *Ifng* and *Il12* transcripts were reduced in the lungs of *Bhlhe40*-deficient mice infected with *M. tuberculosis*. Deletion of Bhlhe40 in either T cells or CD11c+ cells resulted in a similar phenotype of increased mycobacterial burden and early susceptibility, increased IL-10, and decreased IFN-γ. This susceptibility phenotype to *M. tuberculosis* infection resulting from Bhlhe40 deletion was specifically a result of IL-10 action because all these effects were abrogated in mice doubly deficient in both IL-10 and Bhlhe40. Using chromatin immunoprecipitation sequencing in in vitro differentiated T_H1 cells and GM-CSF–driven bone marrow–derived myeloid cells, Huynh et al. (2018) identified a Bhlhe40 binding site at 6 kb relative to the transcriptional start site of *Il10* in both datasets. This site coincided with an evolutionarily conserved region at 6.45 kb previously identified as an enhancer element in T_H2 cells where AP-1 (Jones and Flavell, 2005) and IRF4 (Ahyi et al., 2009) have been shown to bind. These data provide evidence for Bhlhe40 directly repressing *Il10* transcription in both T cells and myeloid cells by binding a downstream cis-regulatory element. The identity of the factor that induces *Il10* transcription in the absence of Bhlhe40 in this model is unknown and may be one of many candidate enhancers previously described (Gabrysova et al., 2014).

Interestingly, the transcription factor c-Maf, shown to bind the *Il-10* locus and induce *Il10* expression, not only has a binding site in the *Il10* proximal promoter but more recently another has been demonstrated around a 6-kb site in the *Il10* locus



Bhlhe40 and c-Maf have opposing effects on *Il10* gene expression: Left: Positions of Bhlhe40 and c-Maf binding at the *Il10* gene locus. Right: A schematic of Bhlhe40 and c-Maf effects on each other's expression as well as the expression *Il10* and *Il2* genes.

(Gabrysova et al., 2018). Of note, in the same study we reported that *Bhlhe40* was up-regulated and found to have increased activity in the absence of c-Maf in T cells (Gabrysova et al., 2018). Because Yu et al. (2018) found *c-Maf* to be up-regulated in the absence of Bhlhe40 it is tempting to speculate that these transcription factors may interact to achieve opposite effects on *Il10* gene expression (see figure showing that Bhlhe40 and c-Maf have opposing effects on *Il10* gene expression; red, Bhlhe40; green, c-Maf). Huynh et al. (2018) also demonstrated two Bhlhe40 binding sites distal to the *Ifng* gene locus in T_H1 cells but not myeloid cells, suggesting that Bhlhe40 positively regulates *Ifng* directly in T cells as well as indirectly by IL-10's action to suppress IL-12 (Huynh et al., 2018), as was also suggested in the study by Yu et al. (2018). It remains to be determined whether the reduced *Il2* gene expression in T cells from Bhlhe40-deficient mice, reported in several of the studies referred to in this commentary, results from a direct effect of Bhlhe40 at the *Il2* locus, although analysis of Huynh et al. (2018)'s data demonstrates little binding of Bhlhe40 at the *Il2* locus compared with the *Il10* locus. However, there is some binding of Bhlhe40 at the *Il2ra* locus (Huynh et al., 2018) as shown previously by chromatin immunoprecipitation PCR (Miyazaki et al., 2010). Alternatively, Bhlhe40 could potentially still be involved in the remodelling of the *Il2* locus or its effects could be achieved by cooperation or competition with other enhancers of *Il2*. In this context, we have recently reported that c-Maf, although positively regulating *Il10* gene expression, negatively regulates *Il2* gene expression (Gabrysova et al., 2018). Again, it is unclear whether this effect is direct or indirect, especially because NFAT2 showed increased activity in c-Maf–deficient $CD4^+$ T cells (Gabrysova et al., 2018). Thus, it would appear that Bhlhe40 and c-Maf play opposing but complementary roles in $CD4^+$ T cells during an immune response: Bhlhe40 induced downstream of TCR/CD28 signaling acts early to promote the production of IL-2 and later in the context of microbes, either from adjuvants, gut flora, or infection, to induce IFN-γ and inhibit IL-10 in both T cells and $CD11c^+$ cells, thus promoting a T_H1 response. Conversely, c-Maf mainly induced in T cells downstream of cytokine signaling resulting from microbial or pathogen stimulation of antigen-presenting cells, in concert with TCR/CD28 signaling in T cells, acts later to limit IL-2 while promoting IL-10 production and thus controls immune responses to pathogens to limit host damage.

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