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COMMENT

What's old is new again: Batf transcription factors and Th9 cells

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Hubble's law, established by famed astronomer Edwin Hubble, states that the universe is rapidly expanding. Although more figuratively, the same can be said of the rapidly expanding universe of $CD4^+$ T cells. In 1986, Mosmann and Coffman famously published a study postulating that all cytokine-secreting $CD4^+$ T helper (T_H) cells could be characterized as either IFNγ-secreting Th1 cells, or IL-4-producing Th2 cells.[1] With few exceptions, this theory was embraced for the better part of the next two decades. Fast forward to the early 2000's, and it soon became clear that naïve T_H cells could adopt additional cell fates in response to an array of cytokine signals that activate a variety of spatially and temporally regulated signaling pathways. For example, naïve T_H cells recognizing antigen in the presence of TGF-β upregulate Foxp3 and differentiate into induced T regulatory (iTreg) cells that express IL-10 and suppress inflammatory responses.[2] If these same naïve T_H cells, however, sense TGF-β in the presence of other pro-inflammatory cytokines, such as IL-6, IL-1β, and/or IL-23, they upregulate RORγt expression, differentiate into Th17 cells that secrete IL-17A, IL-17F, and IL-22, and incite mucosal inflammation (reviewed in ref. [3]). The discovery of iTreg and Th17 cells spurred the descriptions of additional bona fide T_H cell lineages over the past decade, including Th22 cells that secrete IL-22 in the absence of IL-17A/F, T follicular helper (T_{FH}) cells—that express CXCR5, secrete IL-21 and regulate germinal center reactions—and Th9 cells, which differentiate from naïve T_H cell precursors in the presence of TGF-β plus IL-4, and secrete the pro-inflammatory cytokine IL-9.[4]

Originally identified as a Th2 cytokine that promotes the proliferation of mast cells, erythroid precursors, and myeloid leukemia cells[5, 6] IL-9 is now recognized for roles in anti-helminthic and anti-tumor immunity, as well as allergic inflammation. In 2008, two studies independently demonstrated that TGF-β could subvert the canonical Th2 differentiation program and promote the development of effector T_H cells that express IL-9 and IL-10 in the absence of other lineage-defining cytokines.[7, 8] Since these initial reports, IL-9-expressing Th9 cells have become increasingly recognized for their unique pro-inflammatory functions, particularly at mucosal surfaces such as the gut and lung, and found to contribute to the pathogenesis of severe asthma and inflammatory bowel diseases (IBDs; i.e., Crohn's disease and ulcerative colitis) in both mouse and man.

Despite their recognized significance in mucosal inflammatory responses, important questions have lingered with respect to the signal transduction pathways and transcription factors that specify Th9 cell lineage commitment. A number of cytokine (e.g., IL-1β, IL-25, IL-33, TSLP) and co-stimulatory (OX-40, GITR) receptors have been shown to amplify Th9 cell differentiation.[9] A large transcriptional network involving the canonical Th2-promoting transcription factors, GATA-3 and STAT6, the ETS family transcription factor, PU.1, interferon regulatory factor-4 (IRF4), and the AP-1-associated transcription factor basic leucine zipper ATF-like transcription factor (BATF), have been shown to bind to conserved regulatory elements at the *Il9* locus, thereby stabilizing IL-9 expression and supporting Th9 cell differentiation. More recently, the TL1A-DR3 signaling axis has been implicated in T-cell derived IL-9 production, Th9 development, and mucosal immunity.[9] TL1A is a member of the TNF superfamily encoded by the *TNFSF15* locus and induced in a variety of innate and adaptive immune cells following bacterial exposure or FcγR activation; TL1A promotes pro-inflammatory signaling in immune cells via binding to its cognate receptor, death receptor 3 (DR3; encoded by *TNFRSF25*), and inducing p38 MAP kinase and NF-κB-mediated signaling pathways.[9] Experimental analyses in mouse model systems have revealed that genetic or pharmacological neutralization of TLA1 activity ameliorates, whereas TL1A overexpression exacerbates, IBD phenotypes, leading to the concept that microbe-induced TL1A expression tunes host mucosal immunity and inflammation. Consistent with this, TLA1 expression is upregulated in inflamed mucosal biopsies from IBD patients, and the presence of specific TL1A haplotypes is associated with both risk and disease severity in human IBDs.[10] In 2015, Richard et al. described that TL1A-dependent DR3 signaling intrinsically drives Th9 differentiation and Th9-mediated mucosal pathologies in vivo.[11] This was followed by Thomas et al. who revealed that TL1A induces IL-9 production in human $CD4^+$ Th17 cells, and that IL-9 expression contributes to TLA1-driven IL-22 secretion in memory $CD4^+$ T cells.[12] While these studies provided an intriguing link between TLA1 and Th9 cells in mucosal pathologies, the synchronized interaction of cytokines, lineage-specifying transcription factors, and transcriptional regulatory mechanisms downstream of TL1A signaling in Th9 cells has remained incompletely understood.

In this issue of *Mucosal Immunology*, Michelsen and colleagues have extended their previous observations by identifying an intriguing new mechanism whereby TL1A acts synergistically with TGF-β1 and IL-4 to augment Th9 vigor through the BATF-related transcription factor, BATF3.[13] The authors show that TL1A in the presence of the Th9 inducing cytokines, TGF-β and IL-4, increased IL-9 expression in both mouse and human T_H cells. RNA-seq analyses of Th9 cells stimulated with TL1A (referred to as Th9-TL1A cells) revealed elevated expression of both *BATF* and *BATF3*

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expression, as well as a number of BATF-responsive Th9-associated genes, compared with Th9 cells not receiving TL1A co-stimulation (i.e., Th9 cells). Intracellular flow cytometry analyses confirmed TL1A-dependent upregulation of both BATF and BATF3 expression in mouse and human Th9 cells. Intriguingly, the authors also revealed that TL1A co-stimulation markedly increased the proportion of differentiating Th9 cells that co-express BATF and (Interferon Regulatory Factor 4) IRF4, which are known to form cooperative transcriptional complexes at AP-1/IRF composite sites in the *Il9* locus stabilizing gene expression.[14] Previous studies have reported binding of acetylated histone 3 (AcH3) at *Il9* non-coding sequences (CNS) CNS1 and CNS0, which is thought to facilitate chromatin remodeling and *Il9* promoter activity during Th9 differentiation.[15, 16] Through a series of elegant ChIP assays, the authors revealed that TL1A stimulation of T_H cells significantly enhanced TGF-β/IL-4-driven histone acetylation of *Il9* CNS0 and CNS1, BATF and IRF4 binding to CNS0 and CNS1 and BATF3 binding to CNS1 in the *Il9* locus. Recently, a new conserved regulatory element 25 kB upstream of the IL9 gene (*Il9* CNS25/IL9 CNS18) has been identified that binds both BATF and IRF4, as well as STAT5 and STAT6, and that is critical for IL-9 production in Th9 cells.[17] Thus, it will be of interest to know whether TLA1 modulates BATF, IRF4, or BATF3 binding to CNS25, and whether these interactions also alter *IL9* transcriptional activity in Th9 cells. Nevertheless, these studies support a mechanism by which TLA1-induced BATF and BATF3 expression facilitates the cooperative *trans*-activation of *Il9* expression by BATF, IRF4, and BATF3 in Th9 cells.

The authors went on to show that TLA1-dependent Th9 differentiation in vitro was severely impaired in BATF and BATF3-deficient T cells (Figure 1). Although BATF and BATF3 had similar effects on IL-9 expression, the authors also exposed potentially non-overlapping functions of BATF and BATF3 during TLA1-Th9 differentiation. For example, TL1A not only enhanced IL-9 expression in developing Th9 cells but also promoted IL-10 and IL-13 production. Intriguingly, TL1A-mediated IL-10 and IL-13 upregulation in Th9 cells was strictly BATF-dependent and was unaffected by loss of BATF3. In efforts to identify the TL1A-induced signaling pathways involved in BATF and BATF3 upregulation in Th9 cells, the authors revealed a critical role for canonical NF-κB and STAT6-pathways in BATF and BATF3 expression. This is in contrast to previous studies by Richard et al., who reported that TL1A-driven IL-9 upregulation proceeds through STAT5, but not STAT6.[11] Whether the relative involvement of STAT5 and STAT6 (or other STAT family proteins) downstream of TL1A stimulation in Th9 cells is context-dependent will be an interesting area of future investigation. Collectively, these studies make a clear argument that BATF3 is, in fact, a non-redundant transcriptional regulator of both Th9 differentiation, as well as TL1A-driven inflammatory gene expression.

Finally, to establish the in vivo significance of their findings, the authors employed a *Rag*$^{-/-}$ T cell transfer system to evaluate the pathogenic functions of Th9 cells generated in vitro in the presence or absence of either TL1A stimulation or *BATF3*. The authors showed that Th9 cells stimulated with TL1A prior to in vivo transfer displayed enhanced pro-inflammatory functions, as evidenced by exaggerated intestinal and lung pathology in recipient mice. In general, Th9-TL1A cells accumulated better than Th9 cells, in both peripheral (i.e., spleen) and mucosal (i.e., intestine, lung) tissues, possessed increased proliferative capacity and displayed elevated pro-inflammatory gene expression (IL-9, but not IL-13 or IL-17A). Importantly, increased pathogenic function of Th9-TL1A cells in vivo was mitigated by either administration of an anti-IL-9 antibody, confirming IL-9-dependency, or by loss of *Batf3*. Collectively, these results raise important new concepts in the pathways underlying Th9 cell-mediated mucosal pathologies, which could ultimately be leveraged to improve the understanding and treatment of asthma and IBDs.

While these studies unquestionably identify a novel role for BATF3 in TL1A-mediated increase in Th9 vigor, some inconsistencies between the in vitro and in vivo analyses raise important questions concerning the connection between TL1A-BATF3 expression and Th9-driven mucosal pathologies. For example, the authors showed that neither TL1A stimulation nor BATF3-deficiency had significant effects on T_H cell proliferation or alternative lineage-associated cytokine expression in vitro. However, both TL1A and BATF3 regulated T_H cell proliferation and accumulation in vivo. Furthermore, loss of BATF3 in Th9-TL1A cells had no bearing on IL-9 production in vivo, in contrast to its strong (and selective) effects on promoting IL-9 expression in these same cells in vitro. Similarly, BATF3-deficient Th9-TL1A cells transplanted into *Rag*$^{-/-}$ hosts displayed markedly reduced expression of Th17-

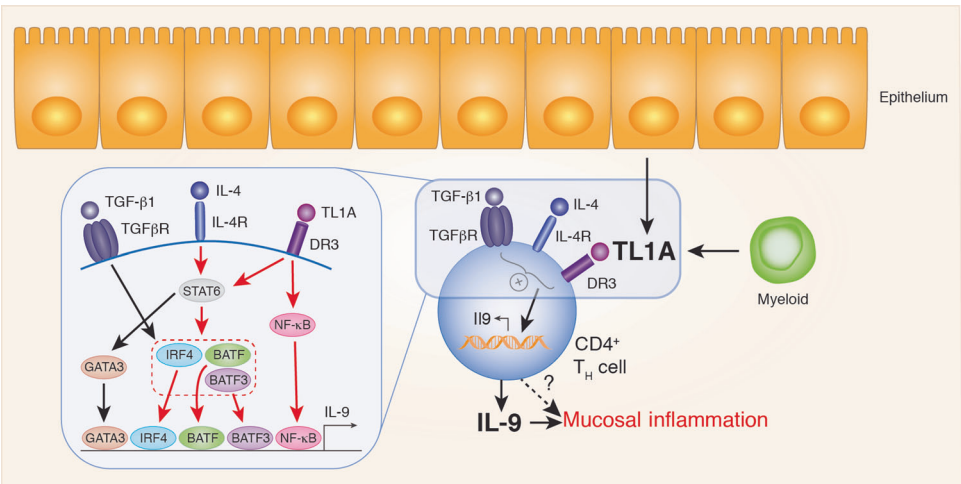

Fig. 1 TL1A enhances the development and inflammatory function of Th9 cells via BATF3. CD4$^+$ T helper (T_H) cells encountering antigen at mucosal surfaces, such as the intestinal tract and lung, are exposed to the TNF family co-stimulatory cytokine TL1A derived from myeloid and epithelial cells. TL1A binds to its cognate receptor, DR3, on T_H cells, which, in the presence of Th9-polarizing cytokines (TGFβ1 and IL-4) promotes enhanced expression of the AP-1 family transcription factors, BATF and BATF3, in a STAT6-dependent manner. Once expressed, BATF and BATF3 bind to regulatory elements in the *Il9* promoter, leading to increased histone acetylation, IRF4 binding and *Il9* gene expression. Accordingly, TL1A enhances, whereas loss of BATF3 represses, Th9-driven mucosal inflammation in vivo

and Th2-associated cytokines, particularly IL-17A and IL-13, which was not evident in the polarized T-cell culture conditions in vitro. Thus, it remains to be determined whether TL1A-dependent BATF3 expression acts in physiological settings as a "specification signal" for Th9 cells, or as a more general amplifier of pro-inflammatory gene expression in multiple T_H cell lineages. Addressing these, and other, important questions will require future efforts to systematically assess how TL1A and BATF3 modify the ever-expanding universe of T_H cell lineages, both in vitro and in vivo.

ADDITIONAL INFORMATION

Conflict of interest: The authors declare that they have no conflict of interest.

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REFERENCES

1. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348–2357 (1986).
2. Chen, W. et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886 (2003).
3. Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M. & Murphy, K. M. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* **24**, 677–688 (2006).
4. Zhu, J. T. Helper cell differentiation, heterogeneity, and plasticity. *Cold Spring Harb. Perspect. Biol*. https://doi.org/10.1101/cshperspect.a030338 (2018).
5. Hultner, L. et al. Mast cell growth-enhancing activity (MEA) is structurally related and functionally identical to the novel mouse T cell growth factor P40/TCGFIII (interleukin 9). *Eur. J. Immunol.* **20**, 1413–1416 (1990).
6. Yang, Y. C. et al. Expression cloning of cDNA encoding a novel human hematopoietic growth factor: human homologue of murine T-cell growth factor P40. *Blood* **74**, 1880–1884 (1989).
7. Dardalhon, V. et al. IL-4 inhibits TGF-beta-induced Foxp3+T cells and, together with TGF-beta, generates IL-9+IL-10+Foxp3(-) effector T cells. *Nat. Immunol.* **9**, 1347–1355 (2008).
8. Veldhoen, M. et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol.* **9**, 1341–1346 (2008).
9. Meylan, F. & Siegel, R. M. TNF superfamily cytokines in the promotion of Th9 differentiation and immunopathology. *Semin. Immunopathol.* **39**, 21–28 (2017).
10. Michelsen, K. S. et al. IBD-associated TL1A gene (TNFSF15) haplotypes determine increased expression of TL1A protein. *PLoS One* **4**, e4719 (2009).
11. Richard, A. C. et al. The TNF-family ligand TL1A and its receptor DR3 promote T cell-mediated allergic immunopathology by enhancing differentiation and pathogenicity of IL-9-producing T cells. *J. Immunol.* **194**, 3567–3582 (2015).
12. Thomas, L. S. et al. The TNF family member TL1A induces IL-22 secretion in committed human Th17 cells via IL-9 induction. *J. Leukoc. Biol.* **101**, 727–737 (2017).
13. Tsuda, M. et al. A role for BATF3 in TH9 differentiation and T-cell-driven mucosal pathologies. *Mucosal Immunol*. (2019). https://www.ncbi.nlm.nih.gov/pubmed/?term=30617301.
14. Humblin, E. et al. IRF8-dependent molecular complexes control the Th9 transcriptional program. *Nat. Commun.* **8**, 2085 (2017).
15. Chang, H. C. et al. The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. *Nat. Immunol.* **11**, 527–534 (2010).
16. Jash, A. et al. Nuclear factor of activated T cells 1 (NFAT1)-induced permissive chromatin modification facilitates nuclear factor-kappaB (NF-kappaB)-mediated interleukin-9 (IL-9) transactivation. *J. Biol. Chem.* **287**, 15445–15457 (2012).
17. Koh, B. et al. A conserved enhancer regulates Il9 expression in multiple lineages. *Nat. Commun.* **9**, 4803 (2018).