BATF2 prevents T-cell-mediated intestinal inflammation through regulation of the IL-23/IL-17 pathway

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Abstract

Inappropriate activation of the IL-23 signaling pathway causes chronic inflammation through the induction of immunopathological Th17 cells in several tissues including the intestine, whereas adequate Th17 responses are essential for host defense against harmful organisms. In the intestinal lamina propria, IL-23 is primarily produced by innate myeloid cells including dendritic cells (DCs) and macrophages (Mφs). However, the molecular mechanisms underlying the regulation of IL-23 production by these cells remains poorly understood. In this study, we demonstrated that BATF2 regulates intestinal homeostasis by inhibiting IL-23-driven T-cell responses. Batf2−/− mice spontaneously developed colitis and ileitis with altered microbiota composition. Batf2−/− mice expressed higher levels of IL-23, but not TNF-α and IL-10, compared with wild-type mice. Moreover, increased numbers of IFN-γ+, IL-17+ and IFN-γ+ IL-17+ CD4+ T cells, but not IL-10+ CD4+ T cells, accumulated in the colons and small intestines of Batf2−/− mice. In addition, RORγt-expressing innate lymphoid cells were increased in Batf2−/− mice. Batf2−/− Rag2−/− mice showed a reduction in intestinal inflammation present in Batf2−/− mice. Furthermore, the high numbers of intestinal IL-17+ and IFN-γ+ IL-17+ CD4+ T cells were markedly reduced in Batf2−/− mice when introducing Il23a deficiency, which was associated with the abrogation of intestinal inflammation. These results indicated that BATF2 in innate myeloid cells is a key molecule for the suppression of IL-23/IL-17 pathway-mediated adaptive intestinal pathology.

Keywords: colitis, gut homeostasis, IL-23/IL-17 pathway, ileitis, innate myeloid cell

Introduction

Inflammatory bowel diseases (IBDs), which include Crohn’s disease (CD) and ulcerative colitis (UC), are characterized by chronic and relapsing-remitting intestinal inflammation (1). The incidence and prevalence of IBD are increasing globally (2, 3), but the etiology of IBD remains poorly understood. An excess of adaptive immune responses driven by pro-inflammatory cytokine IFN-γ-producing CD4+ T (Th1) cells and IL-17-producing CD4+ T (Th17) cells was reported in the intestinal lamina propria of patients with IBD (4). Both Th1 and Th17 responses function in host defense against invading pathogens, while inappropriate effector responses lead to damage of the intestinal mucosa. Because innate myeloid cells instruct the adaptive immune system through antigen presentation and production of anti- and pro-inflammatory cytokines via activation of the pattern recognition receptors signaling pathway (4–6), dysregulation of innate immune responses is
implicated in the pathogenesis of intestinal inflammation. IBD patients have increased numbers of intestinal macrophages (Mφs) with elevated production of pro-inflammatory cytokines in response to gut microbiota and enhanced antigen presentation (7, 8). To maintain intestinal tolerance, the development (9–12) and activation (13–15) of intestinal Mφs are strictly regulated under the homeostatic conditions.

IL-23, a pro-inflammatory cytokine formed by a heterodimer of the IL-23p19 and IL-12p40 subunits, maintains developing T<sub>17</sub> cells (5, 16). Genome-wide association studies (GWAS) indicated that polymorphisms of IL-23/T<sub>17</sub> axis-related genes including IL23R, STAT3 and IL21 are IBD risk factors (17–20). Accordingly, murine studies demonstrated that the IL-23/IL-23 receptor (IL-23R) signaling pathway exacerbated intestinal inflammation by activating innate immune cells (21–25) or T cells (26–28). The beneficial effects of IL-23 during pathogenic bacterial infection in the intestine have also been reported (29–31). In the intestine, IL-23 production is negatively regulated in innate myeloid cells via IL-10R-dependent signaling (14, 15, 32). However, the mechanism underlying the modulation of IL-23 production in intestinal innate myeloid cells is largely unknown.

The transcription factor BATF2, which belongs to the BATF family, was initially characterized as an inhibitor of tumor growth through the suppression of AP-1 activity (33). In addition, BATF2 prevented colonic tumorigenesis and angiogenesis by negatively regulating the HIF-1α/VEGF axis (34). Recent studies have shown that BATF2 is important for appropriate innate immune responses. In Mφs infected with Mycobacterium tuberculosis, BATF2 induced the expression of inflammatory mediators including Tnf, Il12b, Nos2 and Ccl5, by interacting with IRF1 in vitro (35). In tumor-associated Mφs, the expression of Il12b was facilitated through interactions between BATF2 and the p65/p50 heterodimer, leading to the induction of anti-tumor adaptive immune responses (36). We previously reported that BATF2 down-regulated the expression of Il23a by binding directly to c-JUN in Trypanosoma cruzi-infected Mφs and dendritic cells (DCs), thereby suppressing immunopathological T<sub>17</sub> responses (37). However, whether BATF2 exerts its immunoregulatory effect under homeostatic conditions remains unknown.

In this study, we analyzed the function of BATF2 in the maintenance of gut homeostasis. Batf2 deficiency in mice resulted in the development of spontaneous colitis and ileitis. BATF2 mediated the regulation of IL-23/IL-17 axis is required for the prevention of T-cell-mediated intestinal inflammation.

**Methods**

**Mice**

C57BL/6J mice were purchased from Japan SLC (Hamamatsu, Japan). Batf2<sup>−/−</sup> and Il23a<sup>−/−</sup> mice were generated as described previously (37). Batf2<sup>−/−</sup> mice were co-housed with their wild-type littersmates up to 6 weeks of age and subsequently housed single up to the age of 40 weeks. All mice were maintained under specific pathogen-free conditions. All animal experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of Osaka University.

**Reagents**

Dextran sulfate sodium salt was purchased from MP Biomedicals (Kaysersberg, France). The Ca<sup>2+</sup> ionophore A23187, phorbol myristate acetate (PMA) and lipopolysaccharide (LPS; O55: B5) were purchased from Sigma-Aldrich (St Louis, MO, USA). The transcription Factor Buffer Set was purchased from BD Pharmingen (Franklin Lakes, NJ, USA).

**Flow cytometry**

The following antibodies were purchased from BD Biosciences (Franklin Lakes, NJ, USA): 7-AAD, Fixable Viability Stain 510, anti-mouse CD16/32 (clone 2.4.G2), PE-Cy7-conjugated anti-mouse-Ly-6C (clone AL-21), Pacific blue-conjugated anti-mouse CD45R (RA3-6B2), PE-conjugated anti-mouse CD64 (X54-5/7.1), FITC-conjugated anti-mouse Ly6G (1A8), APC-conjugated anti-mouse-CD11c (clone HL3), BV510-conjugated anti-mouse CD3e (145-2C11), BV510-conjugated anti-mouse CD19 (1D3) and BV421-conjugated anti-mouse RO7 (Q31-378), Pacific blue-conjugated anti-mouse CD11b (clone M1/70), Pacific blue-conjugated anti-mouse CD8α (53-6.7), Perpc-Cy5.5- or APC-Cy7-conjugated anti-mouse CD4 (clone CK1.5), APC-conjugated anti-mouse IL-17A (clone TC11-18H10.1), FITC-conjugated anti-mouse IFN-γ (clone XMG1.2), PE-conjugated anti-mouse IL-10 (clone JES5-16E3), FITC-conjugated anti-mouse CD19 (6D5), PE/Cy7-conjugated anti-mouse CD127 (A7R34), APC-conjugated anti-mouse CD196 (29-2L17), FITC-conjugated anti-mouse CD335 (29A1.4), Pacific blue-conjugated anti-mouse CD90.2 (53-2.1), APC-conjugated anti-mouse IL-22 (Poly5164), and PerCP-, FITC- or PE/Cy7-conjugated anti-mouse CD45 (clone 30-F11) antibodies were purchased from BioLegend (San Diego, CA, USA). APC-conjugated anti-mouse Foxp3 antibody (clone 3G3) and FITC-conjugated anti-mouse CD3e (145-2C11) were purchased from TONBO Biosciences (Tucson, AZ, USA). PE-conjugated anti-mouse Igα (ma-6E1) and FITC-conjugated anti-mouse MHCII (M5/14.15.2) were purchased from eBioscience (San Diego, CA, USA). Flow cytometric analysis was performed with a FACSQuant IV flow cytometer (BD Biosciences) with FlowJo software (Tree Star, Ashland, OR, USA). Murine large intestinal cells were isolated with a FACSaria flow cytometer (BD Biosciences). The instrumental compensation was set in each experiment using two-color, three-color, four-color and six-color stained samples.

**Quantitative RT–PCR**

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Manchester, UK), and the RNA was reverse transcribed with Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV) (Promega, Madison, WI, USA) and random primers (Toyobo, Tokyo, Japan) after treatment with RNase I (Promega). Quantitative RT–PCR was performed on a Step One Plus™ Real-Time PCR System (Applied Biosystems) using GoTaq qPCR Master Mix (Promega). All values were normalized to the expression of Gapdh, encoding
glyceraldehyde-3-phosphate dehydrogenase, and the fold difference in expression relative to that of Gapdh is shown. The previously described primer sets for Gapdh, Il23a, Tnf, Il10 and Batf2 were used (37). The amplification conditions were 50°C (2 min), 95°C (10 min) and 40 cycles of 95°C (15 s) and 60°C (60 s).

Isolation of immune cells from the intestine
Murine innate myeloid cells and lymphocytes were isolated from mouse intestines (38) as described previously. Cell surface/intracellular staining of intestinal CD4+ T cells stimulated for 15 s and 60°C (60 s). Batf2 were used (37). The amplification conditions were 50°C (2 min), 95°C (10 min) and 40 cycles of 95°C (15 s) and 60°C (60 s).

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Cytokine analysis
The concentrations of TNF-α, IL-10, IL-23, IL-17A and IFN-γ in culture supernatants were measured with a Cytometric Bead Array (CBA) kit (BD Biosciences).

Histopathological analysis
Large intestines and ileums collected from wild-type, Rag2−/−, Batf2−/−, Il23a−/− mice aged older than 28 weeks were fixed in 4% paraformaldehyde. Paraffin-embedded 5-μm sections mounted on glass slides were used for hematoxylin and eosin (H&E) staining, and tissue histopathology was examined by light microscopy. Each section of large intestines was evaluated for inflammation scores as previously described (41).

Determination of microbiota by deep sequencing
Extraction of bacterial DNA from feces was performed as described previously (42). Libraries were prepared in accordance with the illumina 16s metagenomic sequencing library preparation guide with a primer set (27Fmod: 5′-AGRGTTTGATCMTGGCTCAG-3′ and 338R: 5′-TGCTGCCTCCCTCGTGAGAGTG-3′) targeting the V1-V2 region of the 16s rRNA gene. Then, 251 bp paired-end sequencing of the amplicon was performed on a MiSeq (illumina) using a miSeq v2 500 cycle kit. Raw paired-end sequences were merged using PEAR (http://sco.h-its.org/exelixis/web/software/pear/), and 50,000 reads per sample were randomly selected using seqtk (https://github.com/lh3/seqtk) for further analysis. The processed sequences were clustered into operational taxonomic unit (otu) defined at a 97% similarity cutoff using UCLUST version 1.2.22q. Representative sequences for each OTU were then classified taxonomically using RDP classifier version 2.2, with the greengenes 13_8 database. The bioinformatics pipeline QIIME, version 1.9.1, was used as the informatics environment for all relevant processing of sequencing data and calculation of relative bacterial abundances.

Statistical analysis
Differences between the control and experimental groups were evaluated by Student’s t-test. Differences where P < 0.05 were considered statistically significant.

Results
Innate myeloid cell-specific expression of Batf2 in the large intestine
To determine the role of BATF2 during the steady state, we analyzed tissue expression of Batf2. Higher expression of Batf2 was observed in the spleen, lung, small intestine, cecum and large intestine of wild-type mice (Fig. 1A). We previously demonstrated that Batf2 was expressed in Cd11b+ F4/80+ macrophages (Mφs) in the spleen, but not adaptive lymphoid cells, and that it contributed to the suppression of immunopathological TGFβ1 responses during T. cruzi infection (37). A recent study clearly demonstrated that Batf2 expression induced in Mφs in the lung during infection with M. tuberculosis and Listeria monocytogenes was associated with the pathogenesis of type 1 infectious diseases (43). In addition, this study showed that BATF2 contributed to prevention of type 2 infectious disease caused by Schistosoma mansoni infection in the small intestine. However, the role of BATF2 in the large intestine remains unknown. Therefore, we next attempted to identify Batf2-expressing cell subsets in the large intestine. In the lamina propria of the large intestine, innate myeloid cells, such as Cd11c+hoch Cd103+ Dcs, Cd11b+ Cd64+ Mφs and Cd11b+ Ly6c− monocytes, expressed Batf2, whereas Cd11b+ Ly6c+ neutrophils and Cd11b+ Mhc ii+ Cd64− Dcs did not (Fig. 1B). In accordance with Batf2 expression profiles in the spleen, adaptive immune cells, including T cells, plasma cells and B cells, did not express Batf2 in the large intestine. Thus, Batf2 is highly expressed in some subsets of innate myeloid cells residing in the large intestine as well as the lung and spleen.

BATF2 deficiency induces the development of spontaneous intestinal inflammation with altered microbiota composition
While analyzing the physiological role of BATF2 during T. cruzi infection (37), we found that Batf2−/− mice, but not littermate wild-type mice, developed rectal prolapse over at the age of 28 weeks (Fig. 2A). In addition, almost all of Batf2−/− mice exhibited colon shortening and severe large intestinal pathology with an increased number of inflammatory cells (Fig. 2B and C). Moreover, the infiltration of inflammatory cells was increased in the ileum of Batf2−/− mice (Supplementary Figure 1A), indicating that the lack of BATF2 resulted in the development of spontaneous colitis and ileitis. The altered composition of microbiota, termed dysbiosis, was reported in several animal models of spontaneous colitis including Muc2−/− (44), Mdr1a−/− (45), Il10−/− (46) and Truc (47) mice. Therefore, we analyzed the bacterial composition of the feces from wild-type and Batf2−/− mice by 16s rRNA gene deep sequencing. Class-level fecal bacterial composition was altered between wild-type and Batf2−/− mice at 8 weeks after starting single-housing (Fig. 3). The relative abundance of Bacilli and Epsilonproteobacteria in Batf2−/− mice was higher than that in wild-type mice, whereas Erysipelotrichia...
not affect the development of intestinal innate myeloid cells. We previously showed that IFN-γ-inducible BATF2 in Mψs during T. cruzi infection suppressed the production of IL-23 (37). Therefore, we next examined the expression of cytokine genes in CD11b+ CD64+ Mψs from the large intestines of wild-type and Batf2−/− mice at the age of 8 weeks (Fig. 4B). In LPS-stimulated CD11b+ CD64+ Mψs from Batf2−/− mice, the expression of Il23a, encoding IL-23p19, was higher compared with that in wild-type mice, despite the normal expression of Tnf and Il10. Because the enhanced expression of Il23a in large intestinal CD11b+ CD64+ Mψs from Batf2−/− mice was observed, we further assessed the production of IL-23, TNF-α and IL-10 by CD11b+ CD64+ Mψs in the presence or absence of LPS (Fig. 4C). Without LPS stimulation, CD11b+ CD64+ Mψs from the colons of Batf2−/− mice produced higher amounts of IL-23 than by wild-type cells. In wild-type CD11b+ CD64+ Mψs, IL-23 production was up-regulated in response to LPS, whereas a partial, but not significant, elevation of IL-23 production was observed in Batf2−/− cells. There were no differences in the production levels of TNF-α and IL-10 by wild-type and Batf2−/− CD11b+ CD64+ Mψs stimulated with or without LPS. Thus, BATF2 is responsible for the down-regulation of IL-23 production by innate myeloid cells in the large intestine.

Perturbation of adaptive immune responses is implicated in the pathogenesis of intestinal inflammation in Batf2−/− mice

Several studies demonstrated that IL-23 contributed to T-cell-mediated intestinal pathology by driving the proliferation of T cells, accumulation of IL-17+ CD4+ T cells and induction of IFN-γ IL-17+ CD4+ T cells (26–28). Therefore, we analyzed CD4+ T-cell populations in the large intestinal lamina propria by intracellular staining (Fig. 5A). In Batf2−/− mice, the numbers of IFN-γ+, IL-17+ and IFN-γ+ IL-17+ CD4+ T cells, but not IL-10+ CD4+ T cells, were markedly increased relative to those of wild-type mice. Consistent with the development of ileitis, elevated numbers of effector CD4+ T cells including IFN-γ+, IL-17+ and IFN-γ+ IL-17+ CD4+ T cells were present in the lamina propria of the small intestines of Batf2−/− mice compared with wild-type mice, but no changes in the number of IL-10+ CD4+ T cells were observed (Supplementary Figure 1B). We measured the amounts of IFN-γ, IL-17 and IL-10 in culture supernatants of CD4+ T cells stimulated with or without anti-CD3 antibody (Fig. 5B). In accordance with the intracellular staining analysis, the anti-CD3 antibody-induced secretion of IL-17 and IFN-γ by CD4+ T cells from the colons of Batf2−/− mice was increased compared with wild-type mice, whereas the amount of IL-10 secretion was unaffected. A previous study showed that IL-23 signaling through IL-23R on intestinal T cells inhibited Foxp3+ regulatory T (Treg) cell differentiation, thereby exerting colitogenic activity (28). Although the robust production of IL-23 by CD11b+ CD64+ Mψs was observed, the number of Foxp3+ Treg cells in the large intestinal lamina propria of Batf2−/− mice was similar to that of wild-type mice (Fig. 5C). These results suggest that BATF2 is required for the suppression of effector T-cell responses via a Foxp3+ Treg-cell-independent mechanism. In addition

and Deltaproteobacteria were less abundant in Batf2−/− mice. Thus, BATF2 is required for the inhibition of spontaneous colitis accompanied with dysbiosis.

BATF2 in intestinal CD11b+ CD64+ Mψs negatively regulates the production of IL-23, but not TNF-α and IL-10

Because Batf2−/− mice suffered from chronic intestinal inflammation, we attempted to elucidate the mechanism by which BATF2 modulates intestinal homeostasis. We first analyzed the composition of intestinal innate myeloid subsets (Fig. 4A). To exclude the possibility of inflammation-related changes in composition, we used 8-week-old Batf2−/− mice, which showed no sign of intestinal pathology at the age. The frequencies and total numbers of CD103+ DCs, CD11b+ CD64+ Mψs, monocytes and neutrophils were not altered in the large intestines of Batf2−/− mice, indicating that BATF2 does
Fig. 2. Batf2 deficiency induces spontaneous colitis. (A) Data are representative of the rectal prolapse of wild-type and Batf2−/− mice. (B) Colon length of over 28-week-old wild-type (n = 7) and Batf2−/− (n = 7) mice. *P < 0.05. All graphs show the mean ± SEM. (C) Representative colon sections of 28-week-old wild-type and Batf2−/− mice.

Fig. 3. Altered composition of fecal microbiota in wild-type and Batf2−/− mice. The mean abundance of the listed commensal classes were determined by DNA sequencing of 16S ribosomal DNA in feces from single housed 12-week-old wild-type (n = 9) and Batf2−/− (n = 10) mice (mean values ± SD). *P < 0.05, **P < 0.005, n.s., not significant.
to CD4+ T cells, IL-23 can activate immunopathological RORγt+ innate lymphoid cells (group 3 ILCs: ILC3), which robustly produce TH17-related cytokines including IL-17, IL-22 and GM-CSF (22–25). Compared with wild-type mice, Batf2−/− mice showed an elevated frequency and total cellular number of large intestinal RORγt+ ILCs (Fig. 6A). In addition, an increase in IL-22-producing ILCs was observed in the colons of Batf2−/− mice compared with wild-type mice.
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To determine whether BATF2 limits innate intestinal pathology or adaptive intestinal pathology in the colon, we generated Batf2−/− Rag2−/− mice. Compared to wild-type mice, Batf2−/− mice exhibited worsened colonic histopathology (Fig. 7), which was identified by quantifying the degree of goblet cells loss, mucosa thickening, infiltration of inflammatory cells, ulcers and crypt abscess (41). However, the absence of adaptive immune cells induced by the lack of Rag2 in Batf2−/− mice led to the suppression of spontaneous intestinal inflammation. These findings demonstrated that BATF2 is required for the prevention of spontaneous colitis caused by disrupted adaptive immune responses.
Lack of IL-23 production suppresses the development of IL-17-producing CD4+ T-cell-mediated colitis in Batf2−/− mice

To investigate whether high levels of IL-23 production are involved in the intestinal inflammation in Batf2−/− mice, we analyzed Batf2−/− Il23a−/− mice. In the large intestinal lamina propria of wild-type, Batf2−/−, Il23a−/− and Batf2−/− Il23a−/− mice, the numbers of IFN-γ+, IL-17+, IFN-γ+ IL-17+ and IL-10+ CD4+ T cells were evaluated (Fig. 8A–D). There was no difference in the number of IL-10+ CD4+ T cells in the colons between the four groups. The introduction of Il23a deficiency into Batf2−/− mice resulted in a marked reduction of IL-17+ CD4+ T cells and IL-17+ IFN-γ+ CD4+ T cells in the large intestines, while partially increased numbers of IFN-γ+ CD4+ T cells were observed in Batf2−/− Il23a−/− mice. In accordance with the decreased number of IL-17-producing CD4+ T cells, a low number of infiltrating neutrophils was observed in the lamina propria of the large intestines of Batf2−/− Il23a−/− mice (Fig. 8E). In addition, Batf2−/− Il23a−/− mice showed attenuated large intestinal inflammation, as determined by lower pathological scores (Fig. 8F). These results indicate that the augmented production of IL-23 by innate myeloid cells is associated with the accumulation of IL-17-producing CD4+ T cells, which is implicated in the pathogenesis of intestinal inflammation in Batf2−/− mice.

Discussion

In this study, we identified the mechanism underlying the suppression of IL-23/IL-17 pathway-mediated intestinal inflammation by BATF2 under steady state conditions. In the intestinal mucosa, BATF2, which is constitutively expressed in innate myeloid cells, such as CD11b+ CD64+ Mφs, is required to inhibit IL-23 production, leading to regulation of the IL-23/IL-17 axis that is associated with the pathogenesis of mucosal inflammation in the intestine.

During T. cruzi infection, Batf2 expression is induced in splenic Mφs and bone marrow-derived Mφs by an IFN-γ-dependent mechanism (37). In the intestine, the expression of Batf2 in CD11b+ CD64+ Mφs from the colons of Ifng−/− mice (Batf2/Gapdh: 0.0014 ± 0.00005: our unpublished results) was partially decreased compared with wild-type mice (Batf2/Gapdh: 0.0027 ± 0.0001: our unpublished results). A previous study showed that type I IFNs induced the expression of Batf2 in tumor cells (33). Several functions of type I IFNs in the maintenance of gut homeostasis or pathogenesis of intestinal inflammation have been reported (48). Interestingly, the locus harboring IFNAR, a receptor for type I IFNs, was reported as a susceptibility region for IBD (48). Therefore, it would be interesting to investigate whether type I IFNs induce the expression of Batf2 through IFNAR on intestinal myeloid cells. In addition to Mφs, we observed the constitutive expression of Batf2 in CD103+ DCs from the...
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These cells produce IL-23 in response to flagellin (30). Thus, BATF2 might regulate the Toll-like receptor 5 (TLR5)-induced expression of \( \text{Il23a} \) in intestinal CD103\(^+\) DCs.

In the current study, we showed that CD11b\(^+\) CD64\(^+\) M\(\phi\)s from the colon of \( \text{Batf2}^{-/-} \) mice produced higher amounts of IL-23 than wild-type cells in the presence or absence of LPS. However, IL-23 production by \( \text{Batf2}^{-/-} \) CD11b\(^+\) CD64\(^+\) M\(\phi\)s did not increase after LPS stimulation, while production of IL-10 and TNF-\(\alpha\) was markedly increased. A previous study demonstrated that autocrine IL-10 signaling in intestinal M\(\phi\)s suppresses their IL-23 production during \( \text{Citrobacter rodentium} \) infection and thereby prevents intestinal pathology (32). In addition, M\(\phi\)s from the colon of \( \text{Il10ra}^{-/-} \) mice that spontaneously develop colitis exhibited elevated TLR-induced expression of \( \text{Il23a} \), but not \( \text{Il6} \) and \( \text{Tnf} \) (15). Therefore, colitogenic IL-23 production by intestinal M\(\phi\)s via TLR signaling might be tightly regulated in at least two ways including a BATF2-dependent mechanism and an IL-10-dependent mechanism and disruption of either of the mechanisms leads to perturbation of gut homeostasis.

In the colon of \( \text{Batf2}^{-/-} \) \( \text{Il23a}^{-/-} \) mice, the accumulation of \( \text{T}_1 \) cells was not suppressed completely, whereas numbers of \( \text{T}_17 \) cells and IL-17\(^+\) IFN-\(\gamma\) \( \text{CD4}^{-} \) T cells were markedly diminished. In this context, the severity of spontaneous colitis was attenuated in \( \text{Batf2}^{-/-} \) \( \text{Il23a}^{-/-} \) mice. Thus, IL-17-producing CD4\(^+\) T cells, but not \( \text{T}_1 \) cells, might be linked to the pathology of intestinal inflammation attributed to BATF2 deficiency. Sema4A, a transmembrane protein, is expressed in DCs, activated T cells and \( \text{T}_1 \) cells (49). A previous study showed that it had non-redundant functions: DC-derived Sema4A activated T-cell priming and T-cell-derived Sema4A promoted \( \text{T}_1 \) differentiation (50). We previously demonstrated increased Sema4A expression in \( \text{Batf2}^{-/-} \) bone marrow-derived M\(\phi\)s stimulated with IFN-\(\gamma\) and LPS compared with wild-type cells (37). Thus, increased Sema4A expression in intestinal innate myeloid cells may facilitate both T-cell activation and \( \text{T}_1 \) differentiation in \( \text{Batf2}^{-/-} \) mice.

In addition to the effect on IL-17-producing CD4\(^+\) T cells, IL-23 induces effector functions of ILC3, which produce \( \text{T}_17 \)-related cytokines including IL-17 and IL-22 (51). In the intestines and serum of IBD patients, elevated levels of \( \text{T}_17 \) signature cytokines were observed (52). In this context, IL-23-responsive ILC3 as well as \( \text{T}_17 \) cells were increased (22). Similar to IBD patients, the accumulation of ILC3 and \( \text{T}_17 \) cells was observed in the intestinal lamina propria of \( \text{Batf2}^{-/-} \) mice in this study, but intestinal pathology was abrogated in \( \text{Batf2}^{-/-} \) \( \text{Rag2}^{-/-} \) mice, which harbor ILC3, but not \( \text{T}_17 \) cells. Therefore, BATF2-mediated regulation of IL-23 is essential for host defense against pathogenic bacteria such as \( \text{C. rodentium} \) (39, 53), whereas ILC3 was involved in the IL-23-driven intestinal pathology during infection with \( \text{Helicobacter hepaticus} \) (24). In \( \text{Batf2}^{-/-} \) mice, the number of IL-22-producing ILCs was increased in the colon compared with wild-type mice even in the steady state. Thus, it would be interesting to identify the role of BATF2-dependent suppression of ILC3 responses in gastrointestinal infection in future studies.

Previous studies reported that spontaneous colitis was prevented in \( \text{Il10}^{-/-} \) mice, \( \text{Tcra}^{-/-} \) mice and HLA-B27 Tg rats under germ-free conditions or by microbiota deletion with antibiotics (54). In IBD patients, intestinal CD14\(^+\) M\(\phi\)s robustly produced pro-inflammatory cytokines including IL-23 in response to...
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Fig. 8. Il23a deficiency suppresses spontaneous colitis in Batf2−/− mice by reducing colitogenic effector CD4+ T cells. (A–D) Numbers of IFN-γ* (A), IL-17* (B), IFN-γ and IL-17* (C) and IL-10 (D)-producing CD4+ T cells in the large intestines of 28-week-old wild-type (n = 10), Batf2−/− (n = 12), Il23a−/− (n = 4) and Batf2−/− Il23a−/− (n = 4) mice (mean values ± SD). Graphs represent data pooled from two independent experiments. *P < 0.05, n.s., not significant. (E) Number of CD11b+ Ly6G+ neutrophils in the large intestines of 24- or 28-week-old wild-type (n = 9), Batf2−/− (n = 12), Il23a−/− (n = 5) and Batf2−/− Il23a−/− (n = 4) mice. All graphs show the mean values ± SEM and data pooled from two independent experiments. *P < 0.05, **P < 0.01. (F) Representative colon sections (left panel) and histopathological scores (right panel) of 40-week-old wild-type (n = 8), Batf2−/− (n = 10), Il23a−/− (n = 5) and Batf2−/− Il23a−/− (n = 6) mice. Graphs show the mean values ± SD. ****P < 0.001.
intestinal mucosa, spontaneously developed ileitis, suggest with UC (72) was increased compared with normal intestines. Moreover, SAMP1/Yit mice, such as Nod2 dysbiotic microbiota from spontaneous colitis model mice, previous studies indicated that wild-type mice engrafted with sensitivity to intestinal inflammation (54, 55). In addition, precomposition were observed in many mouse strains with high inflammation. Similar to IBD patients, alterations of microbiota is involved in either the initiation or exacerbation of intestinal activation of innate immune cells against commensal bacteria (56) and TRUC mice (47, 57), developed colitis. In contrast, microbiota from CXCR1-dependent Mϕ-specific Il10ra-deficient mice, which spontaneously develop colitis, did not induce inflammation in the colon of wild-type mice (15). Moreover, SAMP1/Yit mice, Il2−/−mice and Rag1−/−mice transferred with Mdr1-deficient naive T cells exhibited the commensal bacteria-independent development of spontaneous intestinal inflammation (54, 58). In this study, we identified alterations in the fecal microbiota composition of Batf2−/− mice with spontaneous colitis and ileitis, such as increased Bacilli and Epsilonproteobacteria and reduced Erysipelotrichia. Similar to Batf2−/− mice, the patients with ileal CD displayed an elevated abundance of Bacilli (59–61). In addition, Erysipelotrichia was decreased in CD patients (60, 62, 63). A part of bacterial species belonging to the order Campylobacterales (class Epsilonproteobacteria) were increased in IBD patients (64–68) and implicated in the pathogenesis of IBD by promoting production of pro-inflammatory cytokines from intestinal DCs and Mϕs through activation of the NF-κB signaling (66, 68, 69). In the feces of Batf2−/− mice, the relative abundance of Campylobacterales was augmented compared with wild-type mice (data not shown). Therefore, future study should characterize the effect of dysbiotic microbial communities in Batf2−/− mice on the onset and duration of intestinal inflammation.

In this study, we focused on the function of BATF2 in gut homeostasis through the regulation of IL-23 production in myeloid cells. The IL-23 axis is thought to be a potential therapeutic target for IBD. Monoclonal antibodies against p40, which neutralize IL-12 and IL-23, provided therapeutic benefit in clinical trials of CD (5). In addition, IL-23-specific antibodies, which bind to p19 selectively, were safe and effective for the treatment of CD (5, 70). Similar to most CD patients, Batf2−/− mice, in which IL-23 is abnormally produced in the intestinal mucosa, spontaneously developed ileitis, suggesting that BATF2 might be required for the inhibition of IL-23-mediated CD-like intestinal inflammation. IL-23 production by CD14+ Mϕs in CD patients (7, 71) and CD103+ DCs in patients with UC (72) was increased compared with normal intestines. CD4+ T cells co-cultured with CD14+ Mϕs or CD103+ DCs from patients with CD and UC, respectively, produced higher amounts of IL-17 and IFN-γ compared with that by those co-cultured with CD14+ Mϕs or CD103+ DCs of normal intestine (8, 71, 72). GWAS and the IBD exomes browser (https://ibd.broadinstitute.org) indicated that the missense mutations of human BATF2 bear no relationship to IBD. However, the hyperproduction of IL-23 by innate myeloid cells in IBD patients might be associated with dysregulated BATF2 expression possibly related to disrupted epigenetic modification of the BATF2 gene or secondary effects of IBD-related molecules such as IFNAR. Thus, it is important to analyze the expression level of BATF2 in CD14+ Mϕs and CD103+ DCs from IBD patients to gain insights for the development of novel therapeutic interventions for IBD.

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