Basic leucine zipper transcription factor 2 (Batf2) activation is detrimental in Type 1-controlled infectious diseases, demonstrated during infection with *Mycobacterium tuberculosis* (Mtbc) and *Listeria monocytogenes* Lm. In Batf2-deficient mice (Batf2−/−), infected with Mtbc or Lm, mice survived and displayed reduced tissue pathology compared to infected control mice. Indeed, pulmonary inflammatory macrophage recruitment, pro-inflammatory cytokines and immune effectors were also decreased during tuberculosis. This explains that *batf2* mRNA predictive early biomarker found in active TB patients is increased in peripheral blood. Similarly, Lm infection in human macrophages and mouse spleen and liver also increased Batf2 expression. In striking contrast, Type 2-controlled schistosomiasis exacerbates during infected Batf2−/− mice with increased intestinal fibrogranulomatous inflammation, pro-fibrotic immune cells, and elevated cytokine production leading to wasting disease and early death. Together, these data strongly indicate that Batf2 differentially regulates Type 1 and Type 2 immunity in infectious diseases.

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**INTRODUCTION**

Batf2 is a transcription factor that belongs to the basic leucine zipper transcription factor family, which includes Batf and Batf3.1,2 Batf2 is expressed in immune cells such as T cells, B cells,3 macrophages, and dendritic cells.4,5 Batf2 was originally identified as an inhibitor of AP-1 via its interaction with c-JUN in cancer cells.6 We previously reported that Batf2 associates with Irf1 to induce inflammatory responses in IFN-γ and LPS-activated macrophages.6 Knockdown of Batf2 by shRNA in IFN-γ or LPS-activated macrophages caused significant reduction of important early immune response genes (*Tnf*, *Ccl5*, and *Il12b*), including the bacterial effector, killing gene *Nos2*. More recently, another group further elucidated a role for IFN-γ induced Batf2 in mediating IL-17 production and tissue damage during *T. cruzi* infection.7 Additionally, in absence of Batf3, Batf2 compensates for the development of CD103+ Dcs in mice,8 a subset of DCs reported, suppressing helminth-driven immunity through constitutive expression of IL-12.8

In this study, we provide evidence for the regulation of immunity to Type 1 and Type 2 infectious diseases by Batf2. Using human whole blood transcriptomics, we identified elevated expression of BATF2 as an early correlate for tuberculosis (TB) disease progression in adolescents with latent *Mycobacterium tuberculosis* (Mtbc) infection. We further explored the role of Batf2 in a loss of function approach using Mtbc-infected Batf2-deficient mice in comparison to infected wild-type mice. Interestingly, Batf2−/− mice were highly resistant to TB disease exhibiting reduced tissue inflammation, pulmonary histopathology, and subsequently increased survival during acute infection. Mechanistically, we identified Batf2 as a transcriptional inducer of inflammatory responses during Mtbc infection in mice and showed that BATF2 is a predictive biomarker for TB disease in humans in a prospective cohort study in adolescents. Similarly, Batf2 deficiency ameliorated the outcome of murine *Listeria monocytogenes* (Lm) infection by reducing bacterial burden and associated tissue inflammation. In contrast, Batf2 was important to limit untoward immune responses and small intestinal fibro-granulomatous inflammation during murine schistosomiasis. Together, our data reveal a regulatory role of Batf2 on the host immune responses to Type 1 (TB and listeriosis) and Type 2 (schistosomiasis) diseases.
RESULTS

Batf2−/− mice are resistant to the hypervirulent HN878 strain of Mtb with reduced acute lung inflammation

In a genome-wide transcriptomics analysis, we previously reported that Batf2 is highly induced in Mtb-infected and IFN-γ activated macrophages (M1) in vitro.4 Lung alveolar macrophages are the first host cells that become infected by Mtb.5 Thus, we first determined Batf2 mRNA expression in flow-sorted alveolar macrophages (CD11c+‘Siglec-F−‘autofluorescence(high)’) from HN878 Mtb-infected WT and Batf2−/− mice at 3 weeks post-infection. Batf2 mRNA expression was detected in wild-type alveolar macrophages (Fig. 1a). As expected Mtb-infected Batf2−/− mice did not express Batf2 mRNA in alveolar macrophages.

To explore the consequence of a Batf2 deficiency in Mtb infection in vivo, a lethal dose of hypervirulent Mtb HN878 (350 CFU/mouse) was intranasally administered to Batf2−/− mice and control littermates (WT). Subsequent mortality was observed in control littermates, whereas no deaths were observed in Batf2−/− mice (Fig. 1b). Strikingly, 71.4% of the control littermates died during the experiment, whereas all infected Batf2−/− mice survived up to 18 weeks post infection. Kaplan-Meier analysis (Chromotrope Aniline Blue) staining indicating to WT mice (Fig. 1g). Neutrophil infiltration reduced pulmonary bacterial burden (Fig. 1b).

To further analyze the role of Batf2 in TB, mice were infected with a sub-lethal Mtb HN878 intranasal dose (100 CFU/mouse). CFU counts were significantly reduced in Batf2−/− when compared to WT mice at 11 days but similar at 3 weeks post-infection (Fig. 1c). At 3 weeks post infection, Batf2−/− mice resulted in significantly reduced lung weight index, total lung cell numbers, lung pathology score, as well as histopathology (H&E) in the lungs compared to WT mice (Fig. 1d-f, n). Lung inflammation was also quantified by measuring the free alveolar spaces, which demonstrated increased ventilated spaces in Batf2−/− mice, indicative of reduced pulmonary lesions in Batf2−/− mice, compared to WT mice (Fig. 1g). Neutrophil influx into lungs was also reduced in Batf2−/− mice, quantified by tissue-damaging factor myeloperoxidase (MPO) staining (Fig. 1h). Also, T cell recruitment measured by CD3 staining was significantly reduced in Batf2−/− lung sections when compared to WT mice (Fig. 1i). In contrast, C/EBP (Chromotrope Aniline Blue) staining indicating fibrotic tissues were similar between both groups (Fig. 1j). Furthermore, reduced cell numbers and decreased inflammation observed in Batf2−/− lungs was not due to increased apoptosis, but rather attributed to decreased cellular recruitment, hence Caspase-3 staining, a marker for apoptosis10 was similar between both groups (Fig. 1k). INOS, the enzyme producing the anti-mycobacterial effector molecule nitric oxide, was significantly reduced with a concomitant increase in Arg1, a marker for alternatively activated macrophages in Batf2−/− lungs (Fig. 1l, m, o). Together, these results suggest that the presence of Batf2 during sub-lethal Mtb infection has no influence on bacterial burdens in the lungs but strikingly increases detrimental histopathology due to increased pulmonary inflammation and lesion size.

Batf2 induces pro-inflammatory responses in lung recruited macrophages, leading to deleterious inflammation and TB disease progression

To better define cellular infiltration in the lungs, cell populations were analyzed by flow cytometry, at 3 weeks post-infection with 100 CFU/mouse of Mtb HN878. Number and percentages of CD11b+‘F4/80+‘Ly6G− interstitial recruited macrophages, CD11b+‘CD11c+‘MHCII+CD103−‘Ly6C− DC and CD11b+‘Ly6G+ neutrophils in the lungs were significantly lower in Batf2−/− mice compared to WT (Fig. 2a–c and Fig. S1A–C). T lymphocyte population in the lungs and the mediastinal lymph nodes were marginally affected by the absence of Batf2 (Fig. 2d–f and Fig. S1D and E). FACS sorting of interstitial recruited macrophages, CD11b+ DC’s and neutrophils (Fig. S1K, gating strategy) from Mtb-infected wild-type mice showed similar mRNA levels of Batf2 during infection (Fig. 2g). Of importance, recruited interstitial macrophages from Mtb-infected Batf2-deficient mice resulted in reduced transcription of several pro-inflammatory cytokines, as well as chemokines, such as Il1a, Il12a, Cxcl2, and Cxcl3, compared to WT control cells (Fig. 2h–k). In addition, Nos2 was significantly reduced in Batf2−/− compared to WT alveolar and recruited macrophages (Fig. 2i). In contrast, Arg1 and Mrc1, makers for alternatively activated macrophages were significantly increased from sorted alveolar macrophages in Batf2−/− compared to WT controls (Fig. 2m, n), confirming the switch from INOS to Arginase expression in the lung Batf2−/− mice. Non-infected naïve WT control and Batf2-deficient mice displayed similar baseline lung immune cell responses (Fig. S2), with non-detectable levels of MPO, iNOS, and Arg1. These results suggest that Batf2 is involved in early inflammatory responses, with recruiting interstitial macrophages following Mtb infection, increasing early TB disease.

We recently identified a mRNA expression signature of the risk of TB in whole blood that differentiated infected adolescents who progressed to TB disease from adolescents who remained healthy.11 Among the 16 signature genes, BATF2 was significantly increased over time (during progression from latent Mtb infection to TB disease (Fig. 2o). The light green shading represents 99% CI of the spline fit and where this deviates from 0, the data show statistically significant upregulation of BATF2. This analysis confirmed a highly significant upregulation in BATF2 expression over time (p-value: 0.00087) at a false discovery rate of 0.035. Moreover, participants with active TB disease demonstrated striking increased BATF2 mRNA (Fig. 2p) when compared to healthy controls (both QFT and QFT+ P = 0.0004) by RT-PCR. Additionally, Mtb HN878 infection in human monocyte-derived macrophages from healthy donors also showed significant increased BATF2 expression at 48 h post-infection (Fig. 2q).

Comparing to other infections or inflammatory conditions, human whole blood BATF2 expression was significantly increased in patients with Influenza A, B, rhinovirus infection (Public US Cohort GSE 68310) and sarcoidosis (Public UK Cohort GSE 42826) when compared to healthy controls (Fig. S1A). Collectively, this suggests that elevated Batf2 expression is an excellent indicator of host inflammation that also depicts TB disease progression.

Batf2−/− mice are resistant to primary and secondary listeriosis

We further explored whether Batf2 may have a wider importance in Type 1 infectious diseases using experimental murine listeriosis. We uncovered that L. monocytogenes infection induces Batf2 mRNA expression particularly abundant in macrophages followed by the dendritic cells in the spleen (Fig. 3a) and liver (Fig. S3A). In addition, Batf2 mRNA expression was also detected in T cells and B cells to a lesser extent in spleen (Fig. 3a), though absent in liver (Fig. S3A). In naive mice, Batf2 mRNA expression was also higher in macrophages (Fig. S4A), however greatly reduced when compared to Lm-infected mice (Fig. 3a), suggesting Batf2 expression was indeed driven by Lm infection predominantly in macrophages and dendritic cells. Consistent with TB, Lm-infected Batf2−/− mice were also more resistant during high (Fig. 3b) and low-dose (Fig. S3B) infection with increased survival and reduced bacterial burden in the spleen (Fig. 3c) and liver (Fig. S3C), compared to WT mice. Moreover, Lm-infected Batf2−/− mice presented decreased histopathological inflammation, with reduced lesions in spleen (Fig. 3d, e) and liver (Fig. S3D, E) when compared to WT mice, a result consistent with TB lung inflammation. Total spleen cell counts were significantly increased in Lm-infected Batf2−/− mice compared to control mice (Fig. 3f), whereas liver cells were significantly reduced at day 5 after Lm infection (Fig. S3F). At 2-day
post-infection, T cell numbers (CD4+ and CD8+) in spleen (Fig. 3g) and B cells in addition to CD4+ T cells in the liver (Fig. S3G were increased in Batf2−/− mice, whereas myeloid cell populations such as macrophages, dendritic cells, and neutrophils remain unaffected in the spleen (Fig. 3h). Similarly, macrophages and dendritic cells had showed no differences, however, interestingly neutrophils were significantly increased in the liver (Fig. S3H). Importantly, neither the total cell numbers (Fig. S4B) nor immune
cell populations were affected in spleen (Fig. S4C, D) and liver (Fig. S4E, F) of naive mice. Furthermore, IL-12p40 and TNF (Fig. S3i), both important for Lm resistance, were significantly increased in the sera of Lm-infected Batf2−/− mice, as well as nitric oxide, an important effector molecule to clear Lm infection (Fig. 3j).

However, at tissue level reduced IFN-γ, transcripts of Il12b (Fig. S3k) and Il6 (Fig. S3l) were quantified in lung sections per mice (30 µm apart), suggesting that Lm infection directly and/or indirectly control secondary Lm infection. Increased T cell responses, owing to their ability to lodge. We then asked whether Lm infection drives IFN-γ production by macrophages and dendritic cells. Indeed, BATF2 transcripts were increased in splenic dendritic cells (Fig. 3k) whereas Il6 transcripts were decreased in macrophages (Fig. 3m). In liver macrophages and dendritic cells, both Il12b (Fig. S3k) and Il6 (Fig. S3l) transcripts remained unaffected between WT and Batf2−/− mice. As expected, both Il12b and Il6 transcripts were not detected in macrophages and dendritic cells sorted from spleen and liver tissues of naive mice. TNF transcripts in macrophages and dendritic cells were however similar between WT and Batf2−/− mice (Fig. S4j), suggesting a deficiency of Batf2 had no major effect at mRNA level under homeostasis.

We then asked whether Lm infection drives Batf2 mRNA expression in mice. Indeed, Batf2 expression was increased in spleen (Fig. 3n) and liver (Fig. S3m) during listeriosis. Since BATF2 mRNA expression was increased during acute TB disease (Fig. 2o, p) and in Mtb-infected human macrophages (Fig. 2q), we then asked whether Lm infection would also increase BATF2 expression in human macrophages. Indeed, BATF2 mRNA expression was significantly increased in human macrophages at 4 h after Lm infection (Fig. 3o). To directly test whether this increase was specific to Lm, we stimulated macrophages with TNF. At 4 h post stimulation, BATF2 mRNA expression was increased (Fig. 3o), however it was ~25-fold lower when compared to Lm infection, suggesting Lm drives the induction of Batf2 mRNA expression at a greater extent in human macrophages. Furthermore, we asked whether the absence of Batf2 plays a role in memory to Lm infection, which would rapidly clear infection during the secondary challenge. Increased T cell responses, owing to their memory of Lm antigens during the primary challenge, are responsible for enhanced protection during secondary Lm challenge. In Batf2−/− mice, a secondary Lm infection was more rapidly controlled with reduced bacterial burdens in the liver and spleen at 2 and 4 days after infection (Fig. S3n), suggesting that the absence of Batf2 might also enhance the ability of T cells to directly and/or indirectly control secondary Lm infection.
reduction of iNOS expression (Fig. S5C, D). This suggests a reduced ability of small intestinal macrophages, undergoing classical macrophage M1 activation during schistosomiasis in the absence of Batf2. Consistent with our previously defined key role of Batf2 in the promotion of M1 macrophage activation and function. Our present observation supports a necessary role for Batf2 in the host ability to mount an M1 activation profile in the small intestine during acute schistosomiasis. Liver analyses did not show such a
pathological profile in Batf2−/− mice as we observed no altered granulomatous response and a diminished fibrotic response around trapped eggs in the liver of Batf2−/− mice during acute schistosomiasis (Fig. S5E-H). This suggests the small intestine was the primary site of pathology in our model. Notably, despite baseline differences in cellularity and responses (Fig. S6), such a fibro-pathological profile was not apparent in the small intestine of naive Batf2−/− animals (Fig. S7A-F). This suggest a defect in response to infection rather than just an intrinsic defect as a driver of the observed profile in S. mansoni-infected Batf2−/− mice. In support, small intestinal tissue TNF-alpha levels as well as serum TNF-alpha levels were elevated in S. mansoni infected Batf2−/− mice (Fig. S7G, H). Furthermore, histological assessment of small intestinal sections from schistosomiasis-diseased animals showed several opened areas stretching from the lumen to the epithelium leading to loss of intestinal villi in the small intestine of Batf2−/− mice (Fig. 4n). Collectively, these data suggest that the absence of Batf2 results in an exacerbated small intestinal fibrogranulomatous inflammation associated with a wasting disease thus premature death during the Type-2 dominated model of acute schistosomiasis.

Batf2 deficiency results in heightened cellular and cytokine responses in the small intestine during acute schistosomiasis

We next defined the immune responses that associate with a Batf2 deficiency in the small intestine of S. mansoni-infected mice. In the absence of Batf2, there was a significant elevation in the amounts of TNF-α, IL-13, TGF-β and a moderate elevation of IFN-γ, IL-5, and IL-17 (Fig. 5a) in the small intestinal tissue of infected mice. Analyses on the immune cell dynamics in small intestinal tissues showed an expansion of CD4+ and CD8+ intraepithelial lymphocytes (IELs), and CD8+ DCs in percentage (Fig. 5b) and total cell numbers (Fig. 5c). Intracellular cytokine detection in these expanded cells showed an elevated production of pro-fibrotic cytokines, i.e. IL-5, IL-13, and IL-17 by MLN and small intestinal tissue CD4+ and CD8+ T cells (Fig. 5d-i), including the regulatory cytokine IL-10 by MLN CD4+ and CD8+ T cells (Fig. 5i, J), from Batf2-deficient mice when compared to the same cells from littermate controls (Fig. 5d-i). This aligned with elevated cytokine production observed in the small intestinal tissue of S. mansoni-infected Batf2-deficient mice (Fig. 5a). Furthermore, the transcription factors associated with polarization of CD4 T cells (Fig. 5K, L) indicated a general increase of inflammatory response (both Th1 and Th2-related as per the analyzed indicators) rather than a preferential increase of a Th1 helper arm of the immune response in the absence of Batf2. Altogether, these findings demonstrate that Batf2 is required by the host to control T helper mediated inflammatory responses in the small intestine during acute schistosomiasis.

**DISCUSSION**

Here, we show that absence of Batf2 could dampen over-inflamed immunological state leading to resistance against tuberculosis and listeriosis in mice. Deletion of Batf2 in mice displayed enhanced survival rate when compared to control mice that succumbed to hyper-virulent Mtib (HN878) infection. Survival correlated with decreased pulmonary inflammation, revealed by reduced and compact granulomas, which contained similar bacillary loads amongst the groups. We also showed that Batf2 expression in lung macrophages progressively increased following a virulent HN878 strain of Mtib infection, a clinical isolate known to induce inflammation. This showed that Batf2 exerts its inflammatory signature through lung macrophages since flow-sorted macrophages from Mtib-infected Batf2−/− mice had significantly reduced pro-inflammatory cytokine (Cxc2, Cxcl3), cytokine responses (Il1a, Il1b), and killing effector molecule (Nos2), when compared to macrophages from wild-type mice. Interestingly, our findings in Batf2−/− mice mirrored mice lacking the receptor for type 1 IFN, which also resulted in reduced early lung inflammation with smaller lesions. In addition, despite higher bacterial burden (105 CFUs), IFNα-deficient mice were resistant to Mtib infection by decreasing recruitment of inflammatory macrophages, chemo-kines, pro-inflammatory cytokines (Il1a, Il1b, Tnf, Il6) and decreased nitric oxide killing effector function. It seems that Mtib aggregates inflammatory response to develop a persistent replication niche in the host. As TB disease progresses, the accumulation of inflammatory cells drives lung tissue pathology and allows for a permissive state where Mtib can replicate. Indeed, granulocyte depletion significantly extended the survival of Mtib-infected mice and TB susceptibility is determined by the increased accumulation of a permissive monocyte/macrophage population in the lung. The mechanisms of increased resistance of Batf2−/− mice could be explained by reduced recruitment of inflammatory macrophages in the lungs, thereby limiting the numbers and availability of permissive macrophages. Previously, it was reported that Batf3-deficient mice displayed similar survival rates to Erdman strain of Mtib infection compared to wild-type animals. In contrast to Batf3, Batf2 deletion in mice controlled lung pathology and ill-defined granuloma formation that leads to an aggressive TB pathology/damage, which drives the disease progression and subsequent death of the host following HN878 infection. Therefore, the inflammatory response observed during Mtib infection needs to be balanced and an over-inflamed state of immune activation may lead to TB disease and tissue pathology. This balance between pro- and anti-inflammatory signals can be observed spatially within lung granulomas during Mtib infection.

In addition to TB, infection with *Listeria monocytogenes* (Lm) also resulted in increased Batf2 expression, as a function of time
in spleen but transient in liver tissue. Furthermore, flow-sorted splenic cell populations revealed that Batf2 mRNA expression was dominated by macrophages followed by dendritic cells, T cells and B cells during Lm infection in mice. The absence of Batf2 also resulted in an increased production of systemic host protective cytokines (TNF, IL-12) in sera and enhanced recruitment of T and B lymphocytes in mice. This was evident in the observed increased survival rate associated with reduced bacilli loads and decreased spleen and liver histopathology in Batf2−/− mice. Similarly, Lm infection in mice deficient for Batf3 showed enhanced survival due to the depletion of CD8α and CD103+ DCs, obligate entry point populations, which are required for the progressive infection. In contrast to Lm infection, Batf3−/− mice were highly susceptible to Salmonella enterica serovar Typhimurium (ST) infection to their reduced ability to produce inflammatory cytokines (TNF, IL-6, and IL-1α) and chemokines (MIP-1α, MIP-1β).
Batf2 differentially regulates tissue immunopathology in Type 1 and Type 2 infections.

1b) by CD8a DCs, which is required for local CD8+ T cell priming to clear early infection.26 Notably, both Lm and ST are fast proliferating and cytopathic intracellular pathogens, yet the progression of these diseases was different in Batf2−/− mice (amelioration in LM versus aggravation in ST). Considering this, the observed discrepancies in the inflammatory responses between Lm and Mtb infection models are therefore unsurprising, given the differential proliferating profile of both pathogens in the course of an infection, as Lm is rapidly proliferating but Mtb slow growing. Nevertheless, we also found similarities in both infectious models in the absence of Batf2 independently of antigen-presenting cells; these responses were not biased due to gene deletion at homeostasis (Fig. S2, S4, S6). Apart from murine studies, Batf2F expression was decreased during the clinical stage of human lung cancer.27 In contrast to human lung cancer, using South African Adolescent Cohort Study,11 we identified increased Batf2F expression in whole blood of asymptomatic individuals who progressed to active TB disease. Moreover, Batf2 expression was also increased upon Mtb and Lm infection in macrophages derived from healthy human donors. Hence, this shows that the Batf2 expression is detrimental in both Lm and Mtb infections. This matches our conclusions from mouse studies and implies that Batf2F can be used as a predictive marker of disease progression in Type 1 diseases.

Intriguingly, however, Batf2F deficiency aggravated small intestinal fibrogranulomatous responses during acute murine schistosomiasis. This indicates a host requirement of Batf2F to control small intestinal fibrosis in our Th2-dominated setting. Given that excessive small intestinal fibrosis is a widely reported pathology that mediates the burden of several diseases, such as intestinal obstruction,28–30 inflammatory bowel disease31 or ulcerative colitis,32 our finding of a protective role of Batf2 in this context is of potentially wider value. Our data showed a counter-regulatory role of this factor in the advancement of small intestinal fibrosis during schistosomiasis. We also observed higher levels of TNF-α together with disrupted intestinal wall which both point towards a possible role of Batf2 in inducing increased small intestinal permeability during acute schistosomiasis.33–35 but this is still to be addressed experimentally. In fact, our subsequent observations of elevated cytokine release by small intestinal cells together with an elevated recruitment of intestinal cells in Batf2-deficient mice during acute schistosomiasis, further point towards an anti-inflammatory/pathological role of this factor in the small intestine of mice during acute schistosomiasis. Of note, the expansion of small intestinal CD8+ dendritic cells primarily associated with the deleterious effect of Batf2F deficiency during acute schistosomiasis, which does suggest a role for small intestinal CD8+ dendritic cells – and most likely the resulting of elevated CD4+ and CD8+ T cell responses – in the increased small intestinal fibrogranulomatous inflammation reported. The expansion of CD8+ DCs in Batf2−/− mice indicates a regulatory role for Batf2 on small intestinal CD8+ DCs during inflammation.36 Such a regulatory role of Batf2 on inflammatory DCs is consistent with the recently reported inhibitory role of Batf2 on Th17-inducing DCs during T. cruzi infections.37 It remains clear, therefore, that Batf2F deficiency unleashes a pro-inflammatory and cytokine-rich small intestine environment around the trapped S. mansoni eggs, therefore, emphasizing on an anti-inflammatory role of this factor in the small intestine of S. mansoni infected animals. Altogether, our findings therefore strongly argue against a therapeutic target for the Batf2F blockade in the context of Type-2 controlled schistosomiasis in agreement with its recently reported anti-inflammatory role against pathological tissue Th17 responses during T. cruzi infection.

It was shown that Batf2F is expressed in dendritic, monocyte, natural killer, and T cells.38 In our study, we report on a differential inflammatory T cell response in Batf2 knockout mice when compared to WT mice suggesting that some of the observed discrepancies in these cancer models are possibly due to a role of Batf2 in T cells. Consistent with the previously reported higher expression rate of Batf2 by CD8− T cells when compared to CD4+ T cells, our present data reveal a more pronounced inflammatory surge in CD8− cells within the T cells compartment of Batf2-deficient mice during acute schistosomiasis. Further studies on the intrinsic dysregulation of CD8− T cells in the absence of Batf2F independently from antigen-presenting myeloid cells might be needed, for more clarity on the subject. As of now, our present data might support a stronger regulatory need of this factor in CD8− T cells, compared to CD4+ T cells. This might explain the opposing role of the factor in Type-1 and Type-2 diseases, given the differential influence of CD8− T cells in such diseases.39

Overall, whilst a pathogenic role is clear for Batf2F in the context of Type-1 infectious diseases and warrants further assessment of the therapeutic value of inhibiting this factor, Type-2 and Type-17 controlled infectious diseases appears to require Batf2F to tame untoward immune responsiveness and as such might be aggravated by the Batf2F blockade. In the light of our present findings, Batf2F is unprecedentedly presented as a fine and versatile regulator of tissue infectious immunopathologies and caution is therefore recommended in strategies aiming at targeting Batf2F to ameliorate Type-2 infectious diseases.
MATERIALS AND METHODS

Mice
Batf2-deficient mice (Batf2−/−) were generated in 129S6/SvEvTac-derived EDJ22 embryonic stem cells5 and heterozygous mice (Batf2+/−) were purchased by Jackson Laboratories (USA), subsequently intercrossed to generate Batf2−/− and littermate control 129S6/SvEv mice at the Animal Research Facility, University of Cape Town.

Ethics statement
All animal experiments were performed in accordance with the South African National Standard (SANS 10386:2008) and University of Cape Town of practice for laboratory animal procedures. The protocol for Mtb (012/036), Lm (015/037), and Sm (016/027) were approved by the Animal Ethics Committee, University of Cape Town. The human Adolescent Cohort Study1,39 was approved by the Human Research Ethics Committee (045/2005), University of Cape Town.
Mtb, Lm, and S. mansoni infection and determination of burdens in mice

Anaesthetized mice were infected intranasally with 25 µl of viable HN878 Mtb bacilli into each nasal cavity with doses of 100 CFU/mouse for immune response analysis and 350 CFU/mouse for mortality studies. Bacterial loads, histopathological and flow cytometry analyses in lungs of Mtb-infected mice were determined as previously described.40 The lung weight index calculation was performed as a measure of inflammatory infiltration using: square root [(Lung weight in mg/Mouse weight in g)*10]/10. Mice were infected intraperitoneally with Listeria monocytogenes (Lm) with a high-dose of 2 × 10^6 CFU/mouse and a low-dose of 3 × 10^4 CFU/mouse. Tissue burdens, cytokine analysis, and histopathology performed as previously described.41 Briefly, asceptically harvested lungs (Mtb) and liver/spleen (Lm) were homogenized in 0.01% Tween-PBS and 10-fold dilutions were plated on 7H10 (Mtb) and tryptic soy (Lm) agar plates for the determinations of CFUs. Mice were infected percutaneously with 80 live S. mansoni cercariae parasites obtained from infected Biomphalaria glabrata snails (a gift from Adrian Mountfold, York, UK). Eggs were purified from digested sections of liver or ileum of infected animals as previously described.42

Quantitative real-time RT-PCR

Total murine RNA was reverse transcribed by Transcriptor First Strand cDNA Synthesis Kit (Roche) according to manufacturer instructions. Real-time PCR was performed with LightCycler® 480 SYBR Green I Master mix in LightCycler® 480 II (Roche). From the human adolescent cohort study, 43 RNA was extracted from PAXgene tubes and cDNA synthesis was performed with SuperScript II Reverse Transcriptase (Life Technologies). PAXgene tubes and cDNA synthesis was performed with SuperScript™ II Reverse Transcriptase (Life Technologies). According to manufacturer instructions (Thermo Scientific), standard ELISA was then performed on the homogenized samples. From the concentrations obtained by ELISA, each cytokine concentration was normalized to the amount of protein initially measured in a sample. Normalized cytokine concentration = initial cytokine concentration (ng/ml)/total protein content measured per sample in gram.

Isolation of small intestinal cells by enzymatic digestion adapted and optimized studies

The small intestine was cut out from the abdomen and single cells were isolated as previously described with minor modifications.45–47 Briefly, the contents of the excised small intestine were flushed out and the tissue washed in 1X PBS buffer. The tissue was then chopped into fine pieces and then re-suspended into 5 ml digestion buffer solution (220 U/mg Collagenase I and 13 U/mg DNase I in 50 ml DMEM medium supplemented with 5% ICS). The samples were then incubated at 37 °C on a shaker for 30 min. Following incubation, the samples were passed through a 100 µm and 70 µm sieves, then centrifuged at 1200 rpm, for 10 min in 4 °C and re-suspended in 3 ml 1×PBS + 3% FCS. The samples were then supplemented with 1.7 ml isotonic Percoll (9 vol Percoll + 1 vol 10× PBS) and mixed thoroughly by inverting gently. The samples were then centrifuged for 500g, for 10 min at 4 °C without brakes. The supernatant that was carefully removed and the pellets were re-suspended in 5 ml medium (IMDM + 10% ICS and 0.5% Pen-strep) and sieved through a 40 µm sieve. The samples were centrifuged at 1200 rpm, 10 min at 4 °C and then re-suspended in 1 ml of the medium. The cells were assessed for viability and counted using an electronic microscope.

Flow cytometry

The following antibodies were used for flow cytometry analysis: CD4, CD8, CD11b, CD11c, Siglec-F, CD45, IL-4, IL-5, IL-17 purchased from BD Bioscience; F4/80, IL-13 purchased from Affymetrix eBioscience; Ly6G purchased from Sony Biotechnology Inc.; TAAD purchased from Sigma. Cells were re-stimulated non-specifically with a mitogenic cocktail of PMA-Ionomycin and monensin (to

Fig. 4 Batf2 deficiency drives an increased fibrogranulomatous inflammation in the small intestine of S. mansoni infected mice. Control littermates (WT) and Batf2−/− mice were percutaneously infected with 80 live S. mansoni cercariae and were killed at 8 weeks post-infec- tion. Liver and small intestinal tissues were collected from naive and 8 weeks infected WT and Batf2−/− mice. a Batf2 mRNA expression relative to Hprt housekeeping gene by RT-PCR to quantify Batf2 mRNA levels. b Representative survival rate and c body weight change of mice post-infection measured each week up to week 12. d Summary of body weight at week 8 post-infection. Mice were killed at 8 weeks post-infection to determine the number of S. mansoni eggs lodged in the liver (e) and small intestinal f tissues, g the liver weight in grams, the length of the h small intestine (from base of stomach to beginning of cecum) and i colon respectively in cm. j Representative H&E staining of small intestinal sections (scale bar = 200 µm). k Number of small intestinal cells from animals 8 weeks post-infection. L Levels of hydroxyproline and m representative CAB staining of small intestinal sections (scale bar = 200 µm) as a measure of fibrosis. n Representative H&E staining for analysis of small intestinal tissue integrity (scale bar = 200 µm). Error bars denote mean ± SEM. Data shown are representative of one to three independent experiments with a sample size of n = 8–10 mice per group. *p < 0.05, **p < 0.01, and ***p < 0.001 vs WT using one-tailed Student’s t-test, with survival measured using Log-rank (Mantel-Cox) test, and the body weight change measured using Wilcoxon Signed-Rank test. ns not significant
Fig. 5  Batf2 deficiency drives heightened cellular inflammatory responses in the small intestine of S. mansoni mice. Control littermates (WT) and Batf2⁻/⁻ mice were percutaneously infected with 80 live S. mansoni cercariae and were killed at 8 weeks post infection. a Concentrations of small intestinal cytokine levels normalized to mg of tissue were determined using ELISA. Flow cytometry was used to determine percentages (b) and absolute numbers (c) of CD4⁺ intra-epithelial lymphocytes (IEL), CD8⁺ IEL, CD4⁺ CD8⁺ IEL, CD8⁺ dendritic cells, neutrophils (CD11b⁺ Ly6G⁺), macrophages (CD11b⁺ F4/80⁺), and eosinophils (CD11b⁺ Siglec-F⁺), d percentage of IFN-γ CD4⁺, IL-4 CD4⁺, IL-5 CD4⁺, IL-13 CD4⁺, IL-17 CD4⁺ T cells in MLN, e Percentage of IFN-γ CD8⁺, IL-4 CD8⁺, IL-5 CD8⁺, IL-13 CD8⁺, IL-17 CD8⁺ T cells in MLN. f Percentage and g cell numbers of IL-4⁺ CD4⁺, IL-5⁺ CD4⁺, IL-13⁺ CD4⁺, IL-17⁺ CD4⁺ IEL in small intestine. h Percentage and i cell numbers of IL-4⁺ CD8⁺, IL-5⁺ CD8⁺, IL-13⁺ CD8⁺, IL-17⁺ CD8⁺ IEL in small intestine. Error bars denote mean ± SEM. Data shown are representative of one to three independent experiments with a sample size of n = 8–10 mice per group. *p < 0.05, **p < 0.01, and ***p < 0.001 vs WT using one tailed Student’s t-test. ns not significant
block cytokine secretion) for 6–8 h. The cells were stained for the surface, intracellular and intranuclear markers identifying cells, secreted cytokines, and associated transcription factors. The stained cells were then acquired on an LSR Fortessa machine (BD Immunocytometry system) and data were analyzed using Flowjo software (Treestar).

Whole blood RNA-Seq from the Adolescent Cohort Study RNA-Seq was performed on whole blood samples from 46 progressors and 107 non-progressor controls as previously described. BATF2 expression was determined in this cohort by computing a 99% confidence intervals (CI) for the temporal trends by performing 2000 iterations of spline fitting after bootstrap resampling from the full dataset.

Statistical analysis
All data were analyzed using GraphPad Prism v 6.0, a Student’s t-test (two-tailed with unequal variance) or unless otherwise stated in Fig. legends. Means are shown as ± SEM, *P < 0.05, **P < 0.01 and ***P < 0.001 respectively.

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ADDITIONAL INFORMATION
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REFERENCES
1. Guler, R., Roy, S., Suzuki, H. & Brombacher, F. Targeting Batf2 for infectious diseases and cancer. Oncotarget 6, 26575–26582 (2015).
2. Murphy, T. L., Tussiwand, R. & Murphy, K. M. Specificity through cooperation: Batf/IRF interactions control immune-regulatory networks. Nat. Rev. Immunol. 13, 499–509 (2013).
3. Betz, B. C. et al. Batf coordinates multiple aspects of B and T cell function required for normal antibody responses. J. Exp. Med. 207, 933–942 (2010). jem. 20091548.
4. Roy, S. et al. Batf2/IRf1 induces inflammatory responses in classically activated macrophages, lipopolysaccharides, and mycobacterial infection. J. Immunol. 194, 6035–6044 (2015).
5. Tussiwand, R. et al. Compensatory dendritic cell development mediated by Batf-IRF interactions. Nature 490, 502–507 (2012).
6. Z-S, Su et al. Cloning and characterization of SARI (suppressor of AP-1, regulated by IFN). Proc. Natl Acad. Sci. USA 105, 20906–20911 (2008).
7. Kitada, S. et al. BATF2 inhibits immunopathological TH2 responses by suppressing IL22 expression during Trypanosoma cruzi infection. J. Exp. Med. 200, 1313–1331 (2017).
8. Everts, B. et al. Microbial Th13 induction is dispensable for innate and adaptive immunity against low doses of Listeria monocytogenes. Int. Immunol. 11, 325–332 (1999).
9. Cohen S. B., Adams K., Urdahl K. Mycobacterium tuberculosis hijacks alveolar macrophages to translocate from the alveolus to the lung parenchyma. Am. Assoc. Immunol. 196 (1 Supplement) 65A (2016).
10. Porter, A. G. & Janicke, U. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 6, 99–104 (1999).
11. Zak, D. E. & al A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 387, 2312–2322 (2016).
12. Brombacher, F. et al. E-12 is dispensable for innate and adaptive immunity against low doses of Listeria monocytogenes. Int. Immunol. 11, 325–332 (1999).
13. Rothe, J. et al. mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature 364, 798–802 (1993).
14. Ito, S., Ishii, K., Kita, A. & Kliman, D. M. Contribution of nitric oxide to CpG-mediated protection against Listeria monocytogenes. Infect. Immun. 73, 3803–3805 (2005).
15. Ahmed, R. & Gray, D. Immunological memory and protective immunity: understanding their relation. Science 272, 54–60 (1996).
16. De Brossi, R. H. et al. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates Th1 help responses and immunopathology. Immunity 20, 623–635 (2004).
17. Nono, J. K. et al. Host regulation of liver fibrogenic pathology during experimental schistosomiasis via interleukin-4 receptor alpha. PLoS Negl. Trop. Dis. 11, e0005861 (2017).
18. Cao, Y. et al. PLK1 protects against sepsis-induced intestinal barrier dysfunction. Sci. Rep. 8, 1055 (2018).
19. Subbian, S. et al. Early innate immunity determines outcome of Mycobacterium tuberculosis pulmonary infection in rabbits. Cell Commun. Signal 11, 60 (2013).
20. Or dovay, D. et al. The hypervirulent Mycobacterium tuberculosis strain H37Rv induces a potent TH1 response followed by rapid down-regulation. J. Immunol. 179, 522–531 (2007).
21. Dorhoi, A. et al. Type 1 IFN signaling triggers immunopathogenesis in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. Eur. J. Immunol. 44, 2380–2393 (2014).
22. Keller, C. et al. Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. Infect. Immunol. 74, 4295–4309 (2006).
23. Antonelli, L. R. et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive macrophage/mucosal population. J. Clin. Invest. 120, 1674–1682 (2010).
24. Marakalala, M. J. et al. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nat. Med. 22, 531–538 (2016).
25. Edelson, B. T. et al. CD8α+ dendritic cells are an obligate cellular entry point for productive infection by Listeria monocytogenes. Immunology 35, 236–248 (2011).
26. Patel, R. & Sad, S. Transcription factor Batf3 is important for development of CD8+ T-cell response against a phagosomal bacterium regardless of the location of antigen. Immunol. Cell Biol. 94, 378–387 (2016).
27. Zhou, R. J. et al. Decreased SARI expression predicts poor prognosis of Chinese patients with non-small cell lung cancer. Int. J. Clin. Exp. Pathol. 6, 2056–2063 (2013).
28. Strickland, G. T. Gastrointestinal manifestations of schistosomiasis. Gut 35, 1334–1337 (1994).
29. Wilson, M. S. et al. Immunopathology of schistosomiasis. Immunol. Cell Biol. 85, 148–154 (2007).
30. Vanyori, F., Fleming, J. O. & Maiels, R. M. Helminths in the gastrointestinal tract as modulators of immunity and pathology. Am. J. Physiol. Gastrointest. Liver Physiol. 312, G537–G549 (2017).
31. Rieder, F., Fiocchi, C. & Rogler, G. Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. *Gastroenterology* **152**, 340–350 e346 (2017).

32. Gordon, I. O., Agrawal, N., Goldblum, J. R., Fiocchi, C. & Rieder, F. Fibrosis in ulcerative colitis: mechanisms, features, and consequences of a neglected problem. *Inflamm. Bowel Dis.* **20**, 2198–2206 (2014).

33. Ye, D., Ma, I. & Ma, T. Y. Molecular mechanism of tumor necrosis factor-α modulation of intestinal epithelial tight junction barrier. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G496–G504 (2006).

34. Ye, D. & Ma, T. Y. Cellular and molecular mechanisms that mediate basal and tumor necrosis factor-alpha-induced regulation of myosin light chain kinase gene activity. *J. Cell. Mol. Med.* **12**, 1331–1346 (2008).

35. Boivin, M. A. et al. Mechanism of glucocorticoid regulation of the intestinal tight junction barrier. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G590–G598 (2007).

36. Al-Sadi, R., Guo, S., Ye, D. & Ma, T. Y. TNF-α modulation of intestinal epithelial tight junction barrier is regulated by ERK1/2 activation of Elk-1. *Am. J. Pathol.* **183**, 1871–1884 (2013).

37. Kanemaru, H. et al. Antitumor effect of Batf2 through IL-12p40 up-regulation in tumor-associated macrophages. *Proc. Natl Acad. Sci. USA* **114**, E7331–E7340 (2017).

38. Hauptmann, M. et al. Protective and pathogenic roles of CD8+ T lymphocytes in murine Orientia tsutsugamushi infection. *PLoS Negl. Trop. Dis.* **10**, e0004991 (2016).

39. Scriba, T. J. et al. Sequential inflammatory processes define human progression from *M. tuberculosis* infection to tuberculosis disease. *PLoS Pathog.* **13**, e1006687 (2017).

40. Guler, R. et al. IL-4Rα-dependent alternative activation of macrophages is not decisive for *Mycobacterium tuberculosis* pathology and bacterial burden in mice. *PLoS One* **10**, e0121070 (2015).

41. Parihar, S. P. et al. Simvastatin enhances protection against Listeria monocytogenes infection in mice by counteracting Listeria-induced phagosomal escape. *PLoS One* **8**, e75490 (2013).