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Key Roles of MiT Transcription Factors in Innate Immunity and Inflammation

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Abstract

Microphthalmia/TFE (MiT) transcription factors such as TFEB and TFE3, are emerging as key regulators of innate immunity and inflammation. Rapid progress in the field requires a focused update on the latest advances. Recent studies show that TFEB and TFE3 function in innate immune cells to regulate antibacterial and antiviral responses downstream of phagocytosis, IFN- γ , LPS, and adenosine receptors. Moreover, overexpression of TFEB or TFE3 can drive inflammation *in vivo*, such as in atherosclerosis, but in other scenarios, can perform anti-inflammatory functions. MiT factors may constitute potential therapeutic targets for a broad range of diseases; however, to harness their therapeutic potential, sophisticated ways to manipulate MiT factor activity safely and effectively must be developed.

Keywords

TFEB; TFE3; transcription; inflammation; innate immunity; infection

MiT Factors Regulate Innate Immunity

Transcription of pro- and anti-inflammatory genes in the innate immune system is tightly regulated. Appropriate induction of gene expression occurs during infection or injury and is essential for organismal homeostasis and innate immunity. Inappropriate gene expression can drive inflammation, autoimmune disease, and oncogenesis [1,2]. Thus, an assessment of the transcription factors (TFs) that regulate innate immunity and inflammation is essential for understanding homeostasis and pathogenesis.

The MiT family of TFs includes microphthalmia transcription factor (MITF), which binds to enhancer 3 (TFE3), transcription factor EC (TFEC), and transcription factor EB (TFEB)[3]. MiT factors are known to play important roles in melanoma (e.g. MITF [4]) and in autophagy, lysosomal biogenesis, and lipid catabolism (e.g. TFEB and TFE3 [5–10]). Several recent and excellent review articles have highlighted the basic molecular biology of

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MiT factors including their functions during cellular stress, nutrient limitation [3,11–13], and innate immunity [14,15]. However, the rapid pace of progress in this area requires an update on the most recent advances, placing TFEB and TFE3 downstream of important signaling pathways in innate immune cells, such as the toll-like receptor (TLR) and γ interferon (IFN- γ) pathways (Figure 1). These advances also provide a better understanding of the TFEB- and TFE3-mediated regulation of innate immune cell function, including macrophage polarization and cytokine production (Key Figure, Figure 2), and may provide a link between TFEB and TFE3 and the **gut-brain axis** (Glossary and Box 2). In addition to these advances, this review highlights the recently established relevance of TFEB and/or TFE3 in several mammalian inflammatory diseases (Table 1). Indeed, MiT factors are rapidly emerging as key contributors to the regulation of innate immunity and inflammation through several mechanisms.

Novel Regulatory Mechanisms Upstream of MiT Factors in Innate Immune Cells

Overall, two major inputs are known to regulate TFEB and TFE3 in a broad range of cell types and organisms (Figure 1). First, mechanistic target of rapamycin, complex 1 (mTORC1) is a negative regulator that phosphorylates conserved Ser/Thr residues on both factors [10,16–18]. Such phosphorylation enables 14-3-3 protein binding, which restricts TFEB and TFE3 to the cytosol [16]. Second, protein phosphatase 3 (calcineurin) is the most important enzyme that removes mTORC1-mediated phosphorylation, thus allowing TFEB and TFE3 import into the nucleus [19]. Inhibition of mTORC1 or activation of calcineurin are sufficient to trigger TFEB and TFE3 nuclear import [16–19]. Thus, mTORC1 and calcineurin act in opposition: as inhibitor and activator of TFEB function, respectively. Many signaling pathways are known to impact mTORC1 activity under various conditions (e.g. cellular energy status). Regulation of calcineurin, activated by calmodulin and cytosolic Ca^{2+} , is similarly complex [20]. Dual regulation by mTORC1 and calcineurin enables TFEB (and TFE3) to integrate multiple inputs (Figure 1) and feedback loops (Box 1) to affect cellular and organismal physiology in ways that are not trivially predictable. The following sections include a description of recent advances in the upstream mechanisms controlling TFEB and TFE3, with a focus on mammalian phagocytes.

Phagocytosis

A key study showed that phagocytosis is sufficient to activate TFEB. Specifically, phagocytosis of IgG-**opsonized** latex beads activated TFEB in murine RAW264.7 cells and primary bone-marrow derived macrophages (BMDMs), as measured by TFEB nuclear translocation in live cells using fluorescence microscopy and by the induction of TFEB target genes (e.g. *Atp6v1h*, *Ctsb*, *Map1lc3b*) by qRT-PCR [21]. This study showed that protein kinase Syk is activated downstream of the Fc γ receptor (Fc γ R), even if phagocytosis is not complete (a phenomenon called **frustrated phagocytosis** [21]). TFEB activation occurred only in cells that completed phagocytosis, indicating that Syk activation was not sufficient to activate TFEB [21]. By contrast, the same study found that siRNA-mediated silencing of mucolipin 1 (MCOLN1, an important ligand-gated Ca^{2+} channel in lysosomes [22]) prevented phagocytosis-mediated TFEB activation by phagocytosis of opsonized

beads; this suggested that lysosomal Ca^{2+} release was a required step in this process [21]. The functional consequence of TFEB activation resulted in increased killing of subsequently phagocytosed bacteria, as evidenced from bacterial survival in untreated and in *Tfeb*-silenced RAW264.7 cells in vitro [21]. Therefore, MCOLN1-dependent activation of TFEB by phagocytosis (Figure 1) primed macrophages to more effectively kill ingested bacteria. However, the precise mechanism linking phagocytosis to MCOLN1 remains unknown.

Lysosomal damage

Lysosome-permeabilizing agent L-leucyl-L-leucine methyl ester (LLOMe) has been reported to induce TFEB nuclear translocation (visualized via immunofluorescence) in HeLa cells [23]. The mechanism of TFEB activation during lysosomal damage was shown to be mediated by **E3 ubiquitin ligase** tripartite motif containing 16 (TRIM16). For instance, co-localization analysis and affinity precipitation revealed interactions between TRIM16 and **galectin** GAL3, as well as their recruitment to damaged endomembranes in HeLa, HEK293T, and THP-1 cells exposed to LLOMe [23]. Moreover, similar biochemical and cell biology experiments showed that GAL3-mediated TRIM16 recruitment led to the localization of autophagic machinery (unc-52-like kinase 1, ULK1, Beclin1, and autophagy-related gene 16-like 1, ATG16L1) to damaged lysosomes; and furthermore, TRIM16 could interact with mTORC1 subunits and with calcineurin and TFEB [23]. *TRIM16* deletion promoted TFEB nuclear translocation in HeLa cells, in LLOMe-treated and -untreated cells alike, presumably because of defective lysosome quality control in *TRIM16*^{-/-} cells [23]. Thus, GAL3 and TRIM16 emerged as key markers of defective lysosome integrity, inhibiting mTORC1, and triggering both TFEB activation and autophagy of defective organelles [23]. Subsequent work showed similar mechanisms of TFEB activation involving galectins 8 (GAL8) and 9 (GAL9) during lysosomal damage caused by glycyl-L-phenylalanine 2-naphthylamide (GPN) in primary human peripheral blood monocyte-derived macrophages (MDMs, Figure 1) [24]. Moreover, TFEB nuclear translocation in GPN-treated murine BMDMs was partially impaired in *Lgals8*^{-/-} cells relative to wild type (WT) cells [24]. Thus, these studies revealed mechanisms linking lysosomal damage to TFEB activation via galectins and mTORC1 (Figure 1).

Interferon γ

Several studies support a role for TFEB downstream of IFN- γ . For example, during infection of mice with influenza virus, ELISA and qRT-PCR of IFN- γ in sorted alveolar F4/80⁺ CD11c⁺ cells (alveolar macrophages, AMs) showed initial IFN- γ production and decreased expression of macrophage phagocytic receptor with collagenous structure (MARCO) relative to uninfected animals [25]. At later times, IFN- γ secretion was reduced and MARCO expression was increased in the infected animals [25]. RNA-seq of AMs comparing early and late times identified TFEB as a potentially late-activated TF [25]. Moreover, network analysis of the differentially expressed genes suggested that genes encoding IFN- γ , AKT, and TFEB were functionally linked [25]. Consistent with this, IFN- γ -induced decreases in MARCO expression 24h post-infection in primary human alveolar-like monocyte-derived macrophages (AM-MDMs) was prevented by chemical activation of AKT (as evidenced from immunoblot and cell staining) [25]. Moreover, *TFEB* knockdown reversed this effect of AKT activation, suggesting that AKT required TFEB to induce

MARCO during IFN- γ treatment [25]. ChIP-qPCR of the *MARCO* promoter using anti-TFEB antibodies showed that TFEB directly induced *MARCO* transcription in AM-MDMs [25]. Moreover, relative to controls, *TFEB* knockdown versus overexpression in THP-1 cells, caused lower and higher *MARCO* qRT-PCR expression, respectively. Together, these results suggested that influenza virus might downregulate *MARCO* expression through IFN- γ by inactivating AKT and TFEB (Figure 1).

However, a study using murine RAW264.7 and primary peritoneal macrophages (PMs) showed that IFN- γ could activate TFEB [26]. Immunofluorescence and cell fractionation showed that 3h of IFN- γ treatment induced TFEB nuclear translocation [26]. In contrast, PMs from *Hmox1*^{-/-} mice, which lack the gene encoding IFN- γ -induced heme oxidase 1 (HMOX1), showed defective IFN- γ -triggered TFEB nuclear localization, as well as TFEB target expression (immunoblotting of SQSTM1, BECN1, LAMP1, and ATG5); this indicated that HMOX1 was required for IFN- γ to activate TFEB [26]. Moreover, chemical inhibition of HMOX1 prevented IFN- γ -triggered TFEB nuclear translocation in RAW264.7 cells, while chemical elicitation of carbon monoxide (CO), a product generated by HMOX1, reversed the effect of HMOX1 inhibition on TFEB activation by IFN- γ . These results suggested that CO was necessary and sufficient to induce TFEB activation via IFN- γ in vitro [26]. Furthermore, Ca²⁺ imaging and chelation experiments, in addition to chemical and genetic inhibition of calcineurin, showed that Ca²⁺ release and calcineurin were required for CO-elicited TFEB nuclear localization in this model [26]. Thus, this study suggested that TFEB might be activated via IFN- γ through HMOX1-generated CO, Ca²⁺ efflux, and calcineurin in PMs (Figure 1).

The reasons for these conflicting results are not clear. The studies in [25] involved human AM-MDMs and 24h stimulation with 20 IU/ml IFN- γ . Those in [26] involved murine PMs cells and 3h stimulation with 200 IU/ml IFN- γ . It is possible that human and murine macrophages diverge in their regulation of TFEB by IFN- γ , or that IFN- γ dose and timing are important. Nonetheless, a third study examined TFEB activation in murine RAW264.7 cells using 4h treatment with approximately 170 IU/ml IFN- γ , but did not observe TFEB activation [21]. Therefore, more work is required to fully understand the regulation of TFEB via IFN- γ , as well as the functional outcomes of this specific pathway.

Lipopolysaccharide

Lipopolysaccharide (LPS) is a bacterial ligand for several mammalian **pattern-recognition receptors**, such as TLR4, murine Caspase-11, and human Caspase-4 [27]. Treatment of murine BMDM, RAW264.7, or primary **microglial cells** caused TFE3 nuclear import, as well as the expression of TFE3 target LAMP1 (as evidenced from microscopy, cell fractionation and immunoblotting) [28]. ChIP-seq of endogenous TFE3 in LPS-treated RAW264.7 cells compared with controls revealed multiple LPS-specific TFE3 genomic binding sites, and microarray analysis of LPS-stimulated mouse *Tfe3*^{-/-} *Tfeb*^{LysM} double mutant BMDMs revealed a large group of innate immunity genes whose expression was decreased compared with *Tfeb*^{flox/flox} controls [28]. LPS-stimulated *Tfe3* or *Tfeb* single knockout RAW264.7 and BMDM cells produced markedly less TNF- α , IL-6, colony stimulating factor 1 (CSF1, or M-CSF), and 2 (CSF2, or GM-CSF) relative to WT cells,

suggesting non-redundant functions for TFEB and TFE3 [28]. Of note, phosphorylated AKT, mTOR, and mTORC1 targets TFE3, RPS6KB, and EIF4EBP1 failed to show mTORC1 inhibition by LPS via immunoblotting, thus ruling out the possibility that LPS activated TFE3 and TFEB by inhibiting mTORC1. However, the precise mechanism of their activation by LPS remained undefined (Figure 1) [28].

A contemporaneous study showed that LPS treatment of human THP-1 and murine BMDM increased expression of activator of G-protein signaling 3 (AGS3), compared to untreated cells (immunoblotting) [29]. In transfected THP-1 cells sorted by AGS3-GFP expression, AGS3 expression directly correlated with TFEB nuclear localization, as well as target gene expression (*LAMP1*, *CTSD*, *ATP6V1H*) and higher lysosomal proteolysis of DQ-BSA (as evidenced from microscopy, cell fractionation, immunoblotting, and qRT-PCR) [29]. Conversely, in AGS3 knockout *Gpsm*^{-/-} murine BMDM, defective LAMP1 protein expression was reported relative to WT at baseline [29]. Together, these results implicated AGS3 as a positive regulator of TFEB (Figure 1). LPS-triggered AGS3 protein expression was abrogated in *Myd88*^{-/-} and *Ticam*^{-/-} (TRIF) immortalized BMDMs, suggesting that TLR4-mediated LPS detection might be required for AGS3 induction [29]. Furthermore, AGS3 protein expression inversely correlated with phosphorylation of AKT, TSC2, and RPS6KB, suggesting that AGS3 might repress the AKT-mTORC1 signaling pathway [29]. Thus, a model was proposed whereby LPS activated TLR4, inducing AGS3, inhibiting AKT and mTORC1, and thus activating TFEB (Figure 1) [29]. However, TLR4 involvement, as well as the mechanisms for AGS3 induction and for a link between AGS3 and AKT were not directly shown [29].

Recently, studies uncovered a role for **folliculin** (FLCN) in LPS-triggered TFEB and TFE3 activation in murine macrophages. In one key study, LPS stimulation of BMDMs caused a decrease in FLCN protein expression (compared to control cells), concomitant with increased TFE3 nuclear translocation [30]. Conversely, *Flcn* transduction in BMDM prevented TFE3 nuclear translocation following LPS stimulation. Moreover, myeloid-specific deletion of *Flcn* (*Flcn*^{LysM}) resulted in constitutive TFE3 nuclear localization in BMDMs, lysosomal gene induction (*Lamp1*, *Ctsz*, *Ctsd*, *Mcoln1*, *Tpp1*, *Cln1*, qRT-PCR), and lysosomal expansion (LAMP2 immunofluorescence) relative to *Flcn*^{flox/flox} controls [30]. However, *Flcn*^{LysM} BMDMs did not constitutively express higher amounts of TNF- α , IL-6, IL-12, or CCL2 than controls. Nonetheless, exaggerated secretion of these cytokines was reported following LPS or CpG stimulation, suggesting that although not constitutively activated, *Flcn*^{LysM} BMDMs were hyperresponsive to TLR ligands [30]. *In vivo*, *Flcn*^{LysM} mice exhibited spontaneous alopecia, anemia, splenomegaly, and hepatomegaly by 8 months of age; moreover, whole blood analysis and cell sorting showed expansion of the monocyte precursor and neutrophil populations. Histology revealed severe macrophage infiltration in spleen, bone marrow, skin, liver, lymph nodes, and white adipose tissue [30]. Of relevance, these phenotypes were reversed by co-deletion of *Tfe3* in *Flcn*^{LysM} *Tfe3*^{-/-} mice, indicating that activation of TFE3 upon loss of *Flcn* in myeloid cells had dramatic systemic inflammatory effects *in vitro* and *in vivo* [30]. Therefore, it FLCN has been proposed as an LPS-repressed negative regulator of TFE3 that can dampen inflammation in macrophages, although the molecular mechanisms of TFE3 control by FLCN has not been demonstrated.

Additionally, overexpression of *Rragd* in *Flcn*^{LysM} BMDM compared to WT was noted. Indeed, enhanced *Rragd* expression in *Flcn*^{LysM} BMDMs correlated with activated mTORC1 (as evidenced from the expression of phospho-RPS6KB via immunoblotting, as well as from increased cellular proliferation, relative to controls) [30]. Such effect was diminished by *Tfe3* silencing *in vitro*; this suggested the existence of a negative feedback loop where TFE3 induced the activation of its inhibitor mTORC1 via Rragd in mouse BMDMs (Box 1) [30].

The proposed anti-inflammatory role of FLCN through TFE3 inhibition was supported by a contemporaneous study showing that *Flcn* deletion in mouse embryonic fibroblasts (MEFs) caused constitutive TFEB and TFE3 nuclear translocation [31]. Mechanistically, the authors posited that TFEB/TFE3 activation in *Flcn*^{-/-} cells occurred via activation of AMPK (Figure 1) [31]. Chemical activation of AMPK was sufficient to drive TFEB/TFE3 nuclear translocation in MEFs and high expression of G-CSF, IL-6, and VEGF in RAW264.7 cells relative to untreated controls [31]. However, whether the induction of these cytokines in *Flcn*^{-/-} cells was dependent on TFEB/TFE3 was not tested. Overall, although the activation of TFEB and TFE3 by LPS in human and murine macrophages is well established, the precise mechanisms involved remain poorly understood (Figure 1).

Extracellular ATP

Extracellular ATP signals tissue damage and activates the **purinergic receptor** P2X7, which plays important roles in neuroinflammation [32]. A P2X7 chemical agonist was used to activate AMPK in BV-2 transformed murine microglial cells compared with vehicle controls [33]. Biochemical assays showed that P2X7 activation also induced rapid and transient TFEB nuclear translocation and lysosomal expansion (Lysotracker staining), compared with controls [33]. Moreover, chemical inhibition or silencing of AMPK prevented P2X7-triggered TFEB nuclear import, indicating that P2X7 activated TFEB through AMPK (Figure 1) [33]. Thus, TFEB could be activated downstream of P2X7, known to be broadly expressed in myeloid cells. It will be relevant to determine if P2X7 can also regulate TFEB and TFE3 in other innate immune cells, and what the physiological impacts of such regulatory nodes are in health vs disease.

Phagocyte Effector Functions Regulated by MiT Factors

Lysosome/autophagy enhancement

The activation of autophagy and lysosomal genes by TFEB in macrophages was first demonstrated in primary PMs from *Tfeb*^{LysM-OX} transgenic mice overexpressing TFEB in the myeloid compartment [34]. Compared with WT controls, *Tfeb*^{LysM-OX} PMs exhibited 8-fold induction of *Tfeb* and 1.5- to 3-fold induction of TFEB target genes *Sqstm1*, *Lamp1*, and *Map1lc3b* via qRT-PCR [34]. In addition, *Tfeb* overexpression ameliorated the loss of lysosomes caused by cholesterol crystals, compared to WT BMDMs (Lysotracker) [34]. These observations confirmed the observation that TFEB overexpression could be sufficient for increased lysosome biogenesis in murine macrophages, similarly to other cell types, and suggested that TFEB might be relevant to the pathogenesis of atherosclerosis [34]. This area certainly merits further investigation.

Bacterial killing

Nuclear receptor peroxisome proliferator activator receptor α (PPAR- α) is important for the control of *Mycobacterium tuberculosis* growth within macrophages [35]. TFEB was recently identified as a critical mediator of PPAR- α -induced autophagy in *M. tuberculosis*-infected murine BMDMs [35]. In this study, PPAR- α agonists drove TFEB gene expression and nuclear localization, as well as the expression of TFEB target autophagy genes (qRT-PCR of *Uvrag*, *Map1lc3b*, *Vps11*, and *Vps34*), and the induction of autophagy (as evidenced from MAP1LC3 lipidation immunoblotting and puncta formation via fluorescence microscopy) [35]. *Tfeb* siRNA knockdown reduced these effects of PPAR- α activation, thus hampering the control of *M. tuberculosis* survival within BMDM [35]. In contrast to prior findings where TFEB and TFE3 induced IL-6 and TNF- α in LPS-treated or phagocytosing mouse macrophages [28,36], *M. tuberculosis*-infected BMDM with silenced *Tfeb* produced more IL-6 and TNF- α than shRNA controls [35]. Whether this was due to a heightened response to infection or to increased bacterial burden in *Tfeb* shRNA-treated cells was unclear. Nonetheless, TFEB was established as a mediator of PPAR- α -enhanced control of *M. tuberculosis* intracellular growth [35].

An independent study showed that *M. tuberculosis* could inhibit TFEB and autophagy to enhance its intracellular survival [37]. In this study, *M. tuberculosis* induced expression of NF- κ B-dependent micro-RNAs miR-33 and miR-33* in THP-1 cells and murine PMs [37]. qRT-PCR array profiling of PMs revealed that several autophagy genes were differentially regulated upon treatment with miR-33 and miR-33* agonists and antagonists, including *Prkaa1*, which encodes AMPK α and carries a miR-33 target site in its 3' UTR [37]. At baseline, chemical or genetic inhibition of miR-33 enhanced nuclear translocation of TFEB and TFEB target gene expression (*Tfeb*, *Ctsb*, *Lipa*, *Uvrag* by qRT-PCR) in BMDM, compared with controls [37]. Conversely, transfection with miR-33 or miR-33* repressed *Tfeb* expression (qRT-PCR and immunoblot) in WT BMDMs compared with transfection controls, and such repression was abrogated in mouse *Prkab*^{-/-} cells [37]. Therefore, AMPK was required for miR-33/33* repression of TFEB. Furthermore, sorted GFP⁺ AMs from animals infected with GFP-tagged *M. tuberculosis* showed reduced expression of *Tfeb* and its autophagy target genes (*Map1lc3b*, *Lipa*, and *Uvrag*) compared with GFP⁻ cells [37]. Together, these observations suggested that induction of miR-33/33* by *M. tuberculosis* repressed AMPK α . Because AMPK represses mTORC1, it was proposed that AMPK repression might activate mTORC1, thus diminishing TFEB activity and autophagic clearance of *M. tuberculosis* (Figure 1) [37]. Together, these studies support the hypothesis that TFEB may be relevant to *M. tuberculosis* infection in mammalian macrophages, but further study is required to clearly delineate its roles and regulation in this type of infection.

Cytokine production

As mentioned, expression analysis and ChIP showed that TFEB and TFE3 directly controlled the expression of several cytokines and chemokines (e.g. TNF- α , IL-1 α and β , IL-6, M-CSF, and GM-CSF) in murine macrophages infected with pathogenic bacteria or stimulated with LPS [28,36,38]. Additionally, human THP-1 and U937 cells have been documented to secrete IL-1 β in response to high extracellular glucose concentrations [39]. *TFEB* knockdown in these cells impaired IL-1 β secretion compared to siRNA controls [39].

Moreover, high glucose concentrations caused TFEB nuclear translocation, which Ca^{2+} imaging, chemical inhibitors, and genetic knockdowns showed was due to lysosomal Ca^{2+} release and calcineurin activation [39]. However, whether these effects were mediated through alterations in glucose-induced **pyroptosis** or if they represented a novel mechanism for IL-1 β processing and secretion remains unknown. Nonetheless, these findings may have important implications for a putative roles of macrophage TFEB during hyperglycemia.

Macrophage polarization

Several studies indicate that TFEB and TFE3 are important for driving macrophages to a **pro-inflammatory “polarized state”** (also called “classical activation” and “M1”)[40]. As mentioned, *Flcn* deletion can induce TFE3 activation and have a polarizing effect on macrophages [30]. Likewise, myeloid cell-restricted deletion of late endosomal/lysosomal adaptor, MAPK and mTOR activator 1 (LAMTOR1, Figure 1) in *Lamtor1*^{LysM} mice can cause constitutive TFEB activation in macrophages, as evidenced from TFEB immunofluorescence and target gene and protein *Lamp1*, *Ctsd*, and *Map1lc3b* expression in *Lamtor1*^{LysM} BMDM compared to *Lamtor1*^{fllox/fllox} cells [38]. Moreover, LPS-stimulated *Lamtor1*^{LysM} BMDMs have shown enhanced secretion of IL-6 and TNF- α compared with WT, an outcome that was partially suppressed by knockdown of *Tfeb* [38]. Of relevance, ChIP-PCR from RAW264.7 cells showed TFEB recruitment to the *Il6* and *Tnf* promoters after treatment with LPS and mTORC1 chemical inhibitor Torin 1 [38]. Moreover, *Lamtor1*^{LysM} AMs secreted more IL-6 and TNF- α than WT after LPS stimulation *in vivo*, and *Lamtor1*^{LysM} animals were more susceptible than WT to weight loss and death caused by **bleomycin**, in an **acute respiratory distress syndrome** mouse model [38]. As such, *Lamtor1*^{LysM} macrophages displayed stronger responses to pro-inflammatory stimuli than WT, and this effect was at least partially mediated by TFEB.

A separate study found that treatment of murine PMs with murine breast or lung tumor-conditioned medium repressed *Tfeb* gene and protein expression compared with controls [41]. TGF- β neutralizing antibody and recombinant murine TGF- β experiments using murine PMs showed the TFEB-repressing activity to be TGF- β . Moreover, chemical inhibitor experiments showed that TGF- β required ERK signaling to repress TFEB in PMs [41]. To test the role of TFEB in the macrophage **anti-inflammatory polarized state** (i.e. “alternative activation” or “M2”), *Tfeb* was silenced in PMs treated with IL-4 or tumor-conditioned media. *Tfeb* knockdown cells showed increased expression of *Arg1* and *Chil3* (used as markers of the M2-like state), concomitant with decreased expression of suppressor of cytokine signaling 3 (*Socs3*) [41]. Moreover, *Tfeb* knockdown cells showed decreased phosphorylation of signal transducer and activator of transcription 3 (STAT3) [41]. These results suggested that TFEB might repress the M2-like state via induction of *Socs3* and inactivation of STAT3, and that repression of TFEB might license M2 polarization in PMs. Moreover, TFEB activation by chemical or genetic means in IL-4- or tumor-conditioned media-treated PMs decreased *Arg1* and *Chil3* expression compared to untreated cells. And, *Tfeb*^{LysM-OX}-driven macrophage-specific TFEB overexpression correlated with diminished breast cancer tumor growth in *in vivo* bone marrow transplant experiments, compared with WT macrophages [41]. Thus, in tumor-associated macrophages, TGF- β was reported to promote M2 polarization via ERK-mediated repression of TFEB, thus inhibiting M1

polarization and promoting an M2 state. This may have relevant implications for cancer immunotherapy, as M2-like tumor-associated macrophages are thought to limit treatment efficacy for a variety of cancers [41].

Additional support for the M1-polarizing role of TFEB was provided by a study using the anti-malarial drug **chloroquine** (CQ) to increase lysosomal pH [42]. CQ has been ascribed anti-tumor properties in mice and humans [43]. In one study, CQ treatment of murine M2-polarized BMDM drove their M1 polarization, as evidenced from flow cytometry markers IFN- γ , IL-12p40, TNF- α , CD80, CD86, MHC-II, in addition to NO production [42]. Immunoblot and fluorescence microscopy revealed activation of p38 MAPK, NF- κ B, and TFEB by CQ in murine RAW264.7 and M2-BMDM cells [42]. In addition, chemical and genetic inhibition of NF- κ B or p38 MAPK (but not of TFEB) prevented the induction of iNOS and the repression of arginase 1 (ARG1) by CQ (assessed via flow cytometry), suggesting that the NF- κ B and p38 MAPK pathways drove M1 polarization. In contrast, **Seahorse™** experiments showed that the switch from **oxidative phosphorylation** to **lactic fermentation** characteristic of M1 polarization was not affected by NF- κ B or p38 inhibition, but rather, was prevented by *Tfeb* silencing [42]. *In vivo*, mice bearing hepatocarcinoma ascites were injected intraperitoneally with CQ-treated RAW264.7 cells, which resulted in reduced numbers of **myeloid-derived suppressor cells** and **Tregs** at the lesion compared to mice that received PBS-treated cells (assessed by flow cytometry) [42]. Moreover, *Tfeb* siRNA abrogated this effect, demonstrating that TFEB was required for CQ to induce macrophage-dependent antitumor activity *in vivo* [42]. This study concluded that TFEB could promote an M1-polarized state by promoting a metabolic switch to lactic fermentation, rather than through cell fate determination [42].

Antiviral responses

Type I IFNs are major signals in the antiviral response. TFEB can drive the constitutive induction of type I IFN response gene *Ifit1* in *Trex1*^{-/-} murine MEFs, which present high concentrations of cytosolic DNA (Box 1) [44]. Thus, TFEB has been proposed as an anti-viral factor. Consistent with this view, a separate study using primary human macrophages found that HIV-1 infection could activate TFEB via TLR8 (Figure 1, [45]). In this study, HIV-1 infection at early time points in human MDMs, induced autophagy (SQSTM1 degradation and MAP1LC3 lipidation by immunoblot) and inhibited autophagy at later time points [45]. At early times, TFEB translocated into the nucleus, and induced expression of its target genes *UVRAG*, *ATG9B*, and *MCOLN1* (qRT-PCR) compared to uninfected cells [45]. Moreover, *TFEB* shRNA prevented SQSTM1 degradation, MAP1LC3 lipidation, and target gene induction by HIV-1 infection, while viral transduction of *TFEB* caused the opposite effects [45]. Of relevance, shRNA of *TLR8* prevented TFEB nuclear translocation during HIV-1 infection and abrogated downstream gene transcription and autophagy induction; this suggested that viral detection via TLR8 was required for TFEB activation [45]. Furthermore, infection with Nef HIV-1 -- lacking the negative factor gene *Nef* -- produced the same extent of TFEB activation and autophagy induction as infection with WT HIV-1; however, while at later time points the WT virus shut down autophagy, activation of TFEB and of autophagy persisted in the Nef HIV-infected cells [45]. Thus, the study proposed that TLR8 could mediate initial HIV-1 recognition in human macrophages to

activate TFEB and downstream autophagy; at later times, Nef appeared to impair autophagy, presumably to allow virion assembly and productive replication [45]. Future studies are warranted to further assess these observations.

TFEB has also been implicated in the restriction of adeno-associated viruses (AAV). AAV are attractive vectors for gene therapy due to their safety profile [46]. However, to deliver cargo DNA to the nucleus, AAV must overcome several cellular barriers to gene delivery, including cellular degradation and clearance pathways. In HeLa cells, AAV2 infection was reported to activate TFEB compared with uninfected cells (as evidenced from immunofluorescence and qRT-PCR of *MAP1LC3B*, *LAMP1*, *HEXA*, and *SQSTM1*) [46]. Moreover, compared with control cells, chemical activation of TFEB reduced AAV2-mediated transduction while *TFEB* siRNA enhanced it, suggesting that TFEB could pose as a cell-intrinsic barrier to AAV infection [46]. Thus, TFEB inhibition might constitute a promising approach to potentially increase the efficiency of AAV-mediated gene therapies. Collectively, these studies suggest that TFEB can also perform anti-viral functions in macrophages, including the induction of autophagy and the expression of antiviral genes, and it will be interesting to further follow this area of investigation.

Dendritic cell migration

Dendritic cells (DCs) are antigen-presenting cells that when activated (or ‘matured’) migrate to draining lymph nodes to present antigens to T cells. LPS activates DCs, causing them to migrate faster and to follow chemokine gradients [47]. Compared with WT cells, tamoxifen-induced *Tfeb* deletion in primary bone-marrow derived DCs (BMDCs) from *CAG-Cre-ERT* +/- *Tfeb*^{flox/flox} mice resulted in defective LPS-induced speed of migration in microfabricated channels visualized by light microscopy [48]. However, *Tfeb* deletion did not affect LPS-induced DC maturation *per se* (measured by flow cytometry of CD86 and CD40). Conversely, TFEB activation using mTORC1 chemical inhibitor Torin 2 increased migration but did not increase expression of CD86 and CD40 relative to controls [48]. Thus, TFEB appeared to be important for the induction of the migratory phenotype but dispensable for DC maturation. As DC maturation entails **macropinocytosis** downregulation [47], it was proposed that decreased macropinocytosis could lead to mTORC1 inhibition due to decreased cellular nutrient uptake [48]. Inactive mTORC1 would cause TFEB to translocate to the nucleus, thus driving the expression of *Mcoln1*, and further TFEB activation in a Ca²⁺-mediated feedback loop (Box 1). Accordingly, chemical inhibition of macropinocytosis increased TFEB nuclear translocation and *Mcoln1* expression in immature DCs, compared with treatment controls [48]. The authors concluded that LPS could induce DC maturation, reducing macropinocytosis and nutrient intake, and thus, inactivating mTORC1. mTORC1 inactivation resulted in TFEB activation, which was necessary for the induction of increased migratory speed. However, the mechanism linking TFEB to increased speed remained undefined [48].

Concluding Remarks

The emerging picture is that TFEB and TFE3 can modulate innate immunity and inflammation by direct mechanisms, controlling the transcription of inflammatory mediators

(Key Figure, Figure 2, panel a); and by indirect mechanisms, controlling cellular processes (e.g. metabolism, autophagy, and secretion) that impact microbial infection, organismal metabolism, and inflammatory signaling both locally and systemically (Key Figure, Figure 2, panel b). The large number of cellular processes under TFEB control, as well as the many feedback and feedforward mechanisms involved in TFEB regulation, makes it difficult to predict the organismal effects of perturbations to this regulatory network. *In vivo* experiments illustrate how TFEB can perform pro-inflammatory or anti-inflammatory functions, depending on tissue and context (e.g. Table 1).

Several key mechanistic questions remain (see Outstanding Questions). A major focus of future research will undoubtedly be to address the roles of MiT factors in immunometabolism. TFEB and TFE3 may influence inflammatory status and host defense through the interlinked mechanisms of innate immune signaling and metabolic regulation of inflammatory cellular phenotypes, as has been demonstrated in macrophage polarization [42]. Given the roles that TFEB and TFE3 play in lipid catabolism and in mitochondrial homeostasis [7], it is highly likely that they might play important roles in innate immunity through cellular metabolism.

Results in humans and nematodes (box 2) suggest that TFEB activation can reach a threshold above which TFEB activity becomes detrimental. *C. elegans* ceTFEB overexpression has been shown to cause autophagy-dependent lifespan extension of these nematodes [49]. In addition, *C. elegans* maintained on a high-glucose diet has exhibited constitutively activated ceTFEB and high induction of autophagy [50]. However, in this model, glucose-enhanced ceTFEB expression and autophagy was shown to lead to a shorter lifespan autophagy [50]; and furthermore, high glucose has induced the secretion of IL-1 β in U937 human monocytes in a TFEB-dependent manner [39]. Thus, it is reasonable to speculate that inflammatory pathologies such as diabetes might be associated with evolutionarily-conserved TFEB-dependent perturbations and pro-inflammatory mechanisms, although this will require robust testing. Of note, TFEB overexpression alone can cause oncogenic transformation in mice, representing an interesting area of future investigation [51]. Indeed, work to assess MiT factors as putative therapeutic targets for a variety of maladies is currently under way, but extensive effort must be devoted to identifying viable means to modulating these factors in biologically- and clinically-relevant contexts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Acute respiratory distress syndrome

Rapidly progressive pulmonary inflammation

ADF neurons

Sensory *C. elegans* neurons

Anti-inflammatory polarized state

Macrophage activation causing secretion of pro-resolution, anti-inflammatory molecules

ASI neurons

Sensory *C. elegans* neurons

Autophagy

Commonly refers to macroautophagy, in which cells form double-membrane vesicles around portions of the cytosol, which may contain organelles, protein aggregates, or pathogens, for lysosomal degradation

Bleomycin

Fungal antibiotic that causes lung inflammation

Brain/gut/microbiota axis

Term used to describe bidirectional communication between the central nervous system, the gastrointestinal system and the intestinal microbiota

Chloroquine

Synthetic anti-malarial drug

Cry toxin

Pore-forming toxin

Cystitis

Inflammation of the bladder

Dominant negative

allele or construct that inhibits the function of the wild type in a dominant fashion

E-box

The DNA sequence 5'-CACGTG-3'

E3 ubiquitin ligase

E3 ubiquitin ligases recruit ubiquitylation substrates to the E2 ubiquitin transferase

Folliculin

Multi-domain protein that functions as a GTPase activating protein for RRAGC and RRAGD

Frustrated phagocytosis

Scenario where the phagocytic cup is not allowed to close, such as by coating the bottom of the well with scavenger receptor ligand

Gag

Group-specific antigen

Galectins

Proteins that bind to β -galactoside sugar moieties, such as those exposed on membranes of damaged lysosomes

gut-brain axis

Term to describe bidirectional communication between the central nervous system and the gastrointestinal tract

Lactic fermentation

The breakdown of glucose to pyruvate, followed by its reduction to lactate

Lipopolysaccharide

Major lipid component of the outer leaflet of the outer membrane of Gram- bacteria

Lysosome

Membrane-bound organelle in the cytoplasm that contains degradative enzymes

M-box

The cognate sequence for MITF, 5'-TCATGTG-3'

Macropinocytosis

Form of endocytosis; engulfment of large quantities of solutes in an actin-dependent process

Microglia

Glial cells of the central nervous system that function as phagocytes

Myeloid-derived suppressor cells

Type of myeloid cell generated during chronic infection or inflammation that can inhibit T cells and NK cells

Nef

Negative factor, enhances viral titers

Opsonization

Promotion of phagocytic uptake by modification of the surface of the target particle

Oxidative phosphorylation

The consumption of reducing equivalents to generate a proton gradient and the dissipation of such gradient by ATP synthase to generate ATP

Pattern-recognition receptors

Membrane or cytosolic proteins important for sensing molecules derived from microbes

Protease-activated receptors

Transmembrane proteins that include their own ligands, which are activated by proteolysis

Phage display

Technique to express recombinant proteins attached to the tail of a bacteriophage for interaction studies

Phagocytosis

Process by which cells engulf large extracellular particles

Pro-inflammatory “polarized state”

Macrophage activation that leads to the secretion of pro-inflammatory cytokines and chemokines

Purinergic receptor

Plasma membrane ATP receptor

Pyroptosis

Form of pro-inflammatory programmed cell death

Seahorse™

Commercial live-cell metabolic assay platform

T cell help

Mechanism by which T cells stimulate the differentiation of B cells into plasma cells

Tregs

Regulatory T cells, normally identified by the expression of FOXP3

Tryptophan hydroxylase

Enzyme catalyzing the conversion of Trp to serotonin

Vif

Viral infectivity factor, from retrovirus, disrupts function of APOBEC proteins

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Box 1:**Early clues of immunological roles for TFEB and TFE3**

TFEB and TFE3 were linked to the immune system upon their discovery, in a **phage display** screen using cDNA from human B cells to identify proteins that bound to the μ E3 enhancer of the human immunoglobulin heavy chain locus [52,53]. Subsequently, T-cell specific expression of a **dominant negative** construct that inhibited TFEB and TFE3 in transgenic mice caused defective expression of cluster of differentiation 40 ligand (CD40L) compared with WT, leading to a defect in plasma B cell differentiation [12]. Moreover, *in vitro* gel shift and promoter-luciferase assays using primary murine CD4⁺ splenic T cells showed that TFEB and TFE3 bound to **E-boxes** in the *Cd40lg* promoter to induce its expression [12]. Thus, TFEB and/or TFE3 were directly linked to CD40L-mediated **T cell help** to drive B cell differentiation [54].

Subsequent reports uncovered links between TFEB and inflammation. The first study used mice whose bladders were infused with peptide agonists specific for three distinct **protease-activated receptors** (PARs), as a model for **cystitis** [55]. This study used protein-DNA microarrays representing 345 consensus binding sequences to identify activated TFs in nuclear extracts from the inflamed bladder mucosae. The only transcription factor whose binding was activated by all three peptides was TFEB. Immunohistochemical detection of TFEB in inflamed tissue and additional functional assays supported the idea that TFEB was activated downstream of PARs [55]. This was the first time that TFEB was proposed as a potential therapeutic target for inflammatory disorders.

Recently, another study showed that loss of cytosolic DNase three-prime repair exonuclease 1 (TREX1) activated TFEB and lysosomal biogenesis in mouse and human fibroblasts [44]. *Trex*^{-/-} mouse cells exhibited mTORC1 inhibition, measured by immunoblot of phosphorylated S6 kinase (RPS6KB) and EIF4EBP1, constitutive TFEB nuclear localization (by immunofluorescence), and lysosomal expansion (Lysotracker and LAMP1 staining). This was proposed as a trigger for interferon-independent activation of antiviral genes, such as *Ifit1*, *Ifit2*, and *Ifit3*, in *Trex1*^{-/-} mouse fibroblasts via a pathway involving stimulator of interferon genes (STING)-TANK binding kinase 1 (TBK1)-interferon regulatory factor 3/7 (IRF3/7) [44]. However, the molecular mechanisms linking TREX1 to TFEB, and lysosomal biogenesis to the STING pathway, remain unknown [44].

Box 2:**TFEB and host defense in *Caenorhabditis elegans***

C. elegans can recognize infections by bacterial pathogens and induce pathogen-specific transcriptional defense programs independently of Toll-like receptor (TLR) and nuclear factor κ B (NF- κ B) pathways [56]. The first bioinformatic study implicating TFEB in the induction of host defense genes revealed an over-representation of **M-box** in promoters of *C. elegans* genes induced by *Staphylococcus aureus* infection; this suggested potential transcriptional regulation by an MiT factor. *C. elegans* harbors one MiT factor gene, *hlh-30* (*ceTFEB*) and 80% of the host innate immune response was abrogated in *ceTFEB* mutants compared to WT; affected genes included **autophagy** and lysosomal, as well as antimicrobial genes, contributing to infection survival as revealed via RNAi and mutant analyses [36]. GFP-tagged ceTFEB translocated into somatic cell nuclei shortly after infection, suggesting that ceTFEB activation was an early event in the host response [36]. Murine RAW264.7 cells and primary BMDMs showed TFEB nuclear translocation promptly after *S. aureus* infection [36]. Moreover, *S. aureus*-infected RAW264.7 cells with silenced *Tfeb* were impaired in the induction of cytokine and chemokine genes including *Il1b*, *Il6*, and *Tnf* compared to siRNA controls (qRT-PCR) [36]. TFEB was thus deemed an evolutionarily conserved host defense transcription factor. ceTFEB was also implicated in host defense against bacterial pore-forming toxins [57]. Nematodes lacking autophagy by mutation (e.g. *BECLIN1* homolog *bec-1* or *ATG4* double mutant *atg-4.1; atg-4.2*) and intestine-specific knockdown (e.g. *MAP1LC3B* homologs *lgg-1*, *lgg-2*, and *lgg-3*) exhibited defective survival due to intoxication with purified **Cry toxins** made by *Bacillus thuringiensis*, compared with vector-treated controls [57]. Consistent with ceTFEB's known roles in autophagy and host defense [36,49], *ceTFEB* mutants were more susceptible to Cry-mediated killing [57]. Thus, ceTFEB is essential for defense against pathogenic bacteria and isolated pore-forming toxins.

Other studies showed that ceTFEB is essential for behavioral host defense via production of INS-11 in the intestinal epithelium: INS-11 (insulin-like neuropeptide involved in gut-to-nervous system communication) elicits protective behavioral responses in *C. elegans* [58,59]. Enteric infection with *P. aeruginosa* induced an *ins-11* transcriptional GFP reporter in the intestinal epithelium compared with noninfected controls [58]. *In vivo*, mutated *ins-11* eliminated aversive behavior towards *P. aeruginosa*, including derepression of *tph-1* (encoding **tryptophan hydroxylase**) in **ASI** neurons, and of *ins-6* (encoding an insulin-like neuropeptide linked to aversive learning) in **ADF** neurons, compared to WT (via fluorescent transcriptional reporters) [58]. A *ceTFEB* mutation abrogated intestinal induction of *ins-11* by *P. aeruginosa*, increasing *tph-1* and *ins-6* expression and defective aversive learning compared to WT [58]. Using the same *ceTFEB* mutant and *ins-11* fluorescent reporter, an independent study showed that ceTFEB was required for *ins-11* intestinal expression during infection by *P. aeruginosa* or *Serratia marcescens* [59]. During intestinal infection, ceTFEB induces neuropeptide production by the intestine and modulates behavior, and ceTFEB is an essential component of the gut-nervous system axis in nematodes. It will be important to

determine whether TFEB and TFE3 contribute to the mammalian **brain/gut/microbiota axis** [60].

In an RNAi screen [61], RNAi of *dkf-1*/DKF-1 (homolog of mammalian protein kinase D (PrKD) activated by diacylglycerol (DAG) via phospholipase C (PLC) [62]) prevented GFP-ceTFEB nuclear import and ceTFEB target gene induction (e.g. *ilys-2* and *lys-5*, by RT-qPCR) during *S. aureus* infection of *C. elegans* [61]. Accordingly, knockdown of *plc-1* (PLC ϵ) or *egl-30* (G α_q) abrogated ceTFEB nuclear translocation and target gene induction compared with control animals [61]. Chemical inhibition and knockdown experiments also showed that the PLC-PrKD pathway was required for nuclear translocation of GFP- or FLAG- tagged TFEB in stably transfected RAW264.7 cells infected with *S. aureus* or *S. enterica* Typhimurium [61]. Thus, the PLC-PrKD-TFEB pathway is evolutionarily conserved (Figure 1). A second pathway of AMP-activated kinase (AMPK) was recently described: simultaneous deletion of *aak-1* and *aak-2* (encoding AMPK α_1 and α_2 catalytic subunits), decreased nuclear translocation of ceTFEB-GFP during *S. aureus* infection in *C. elegans* relative to WT controls [31]. RNAseq revealed that roughly a sixth of ceTFEB-dependent *S. aureus*-induced host defense genes were dependent on *aak-1* and *aak-2* compared to WT, causing reduced survival from infection for *aak-1;aak-2* mutants relative to WT [31]. AMPK was thus required for full activation of ceTFEB during infection, via an unknown mechanism.

Box 3:**Regulation of TFEB by multiple feedback loops**

Feedback loops are pervasive mechanisms by which a regulatory step can influence the activity of an upstream step [63]. Recent work has uncovered several feedback loops that modulate TFEB activity in a variety of mammalian cell types (Figure I).

TFEB-TFEB positive loop

The *TFEB* promoter includes several **CLEAR motifs**, mediating TFEB auto-regulation in a positive feedback loop in many cell types (e.g. HeLa, human and mouse hepatocytes; Fig. Ia) [7].

TFEB-MCOLN1 positive loop

Activation of MCOLN1 is necessary and sufficient to activate TFEB in many cell types (e.g. HeLa, human hepatocytes, macrophages) mediated by Ca^{2+} and calcineurin [19]. In addition, *MCOLN1* is directly induced by TFEB in these cells [5]. This positive feedback loop (Fig. Ib) may amplify the Ca^{2+} wave initiated from the lysosome, boosting TFEB nuclear import and downstream target gene activation.

TFEB-PGC-1 α /PPAR- α positive loop

TFEB directly activates *PGC1A* and *PPARA* expression in HeLa and hepatocyte cells during nutrient starvation [7]. Conversely, PGC-1 α can bind to the *TFEB* promoter, inducing TFEB expression through PPAR- α [7]. This positive feedback loop (Fig. Ic) induces **β -oxidation** genes and mitochondrial biogenesis in hepatocytes [7] and brain cells [64,65].

TFEB-mTORC1 negative loop

TFEB induces endocytosis in HeLa and HEK293 cells, driving the formation of signaling endosomes, modulating cell signaling, and enhancing the intake of nutrients, resulting in recovery of mTORC1 activity after starvation [66]. In addition, TFEB directly induces the expression of *RRAGD* in HeLa cells, thus enhancing mTORC1 activity [67]. Via these two mechanisms, TFEB participates in a negative feedback loop that tends to restore homeostatic activity (Fig. Id).

TFEB-autophagy/lysosome negative loop

Disruption of autophagy or autophagosome-lysosome fusion leads to TFEB activation in HeLa and hepatocyte cells [6]. TFEB drives the transcription of a large number of autophagy-related genes and induces autophagy, restoring autophagic flux [6]. When autophagic flux is restored by this negative feedback loop, the TFEB-activating signal diminishes and TFEB activity is reduced, returning to homeostatic equilibrium (Fig. Ie).

Figure I. Feedback regulation of TFEB in human cells. (a) TFEB drives expression of the *TFEB* gene. (b) MCOLN1/ Ca^{2+} /calcineurin signaling activates TFEB, driving the expression of MCOLN1. (c) PPAR- α and PGC-1 α drive *TFEB* expression, while TFEB drives the *PPARA* and *PGC1A* expression. (d) TFEB drives expression of *RRAGD* and endocytosis genes, enhancing mTORC1 lysosomal recruitment and activation. Activated

mTORC1 represses TFEB. (e) Lysosomal damage and disruption of autophagy activate TFEB. Activated TFEB drives lysosomal biogenesis and autophagy, diminishing the activating signal. TFEB, transcription factor EB; MCOLN1, mucolipin 1; PPAR- α , peroxisome proliferator activator receptor α ; PGC-1 α , PPAR- γ coactivator 1 α ; mTORC1, mechanistic target of rapamycin, complex 1; RRAGD, Ras-Related GTP-Binding Protein D; ATG, autophagy. Figure created via BioRender (<https://biorender.com/>)

Outstanding Questions

- What are the tissue- and organ-specific functions of TFEB (and other MiT factors) in the context of acute and chronic inflammation and host defense against infection? This information is critical to understanding the tissues that might be affected by therapeutic targeting of individual MiT factors, and to manage off-target effects.
- What are the specific upstream regulatory pathways in each cell type? This question is important, because although many pathways have been identified in different cell types and species, the relevance of each pathway to specific disease conditions and specific cell types is not fully understood.
- Can we develop therapeutically viable strategies to manipulate MiT factors? Investigating this merits attention as MiT factors perform several essential cellular functions in homeostasis and their overactivation can be detrimental.
- How do MiT metabolic functions fit into the bigger picture of immunometabolism? Indeed, MiT factors might be important regulators of innate immunity and inflammation via presumed metabolic functions.

Highlights

- TFEB, TFE3 and related MiT transcription factors are broadly expressed in mammals.
- Under resting or homeostatic conditions, TFEB and TFE3 reside in the cytosol; their activation entails nuclear translocation, which is triggered by many distinct stimuli.
- Recently identified upstream regulation of TFEB and/or TFE3 in innate immune cells includes phagocytosis, lysosome damage, IFN- γ , LPS, and extracellular ATP.
- Recently identified downstream functions of TFEB and/or TFE3 in innate immune cells include autophagy and lysosomal biogenesis, bacterial killing, pro-inflammatory cytokine production, macrophage classical activation, antiviral responses, and dendritic cell migration.
- Multiple feedback loops control TFEB and TFE3, mandating sophisticated approaches to safely control their activities for therapeutic purposes.

Figure 1. Upstream regulation of TFEB in murine and human macrophages.

TFEB (highlighted in yellow) is known to be regulated by a large number of stimuli and pathways in mammalian macrophages. At the center, mTORC1 (red) and calcineurin (green) are the two major post-translational regulators of TFEB. Several upstream pathways feed into the regulation of mTORC1 and calcineurin, as indicated. Dashed arrows indicate unknown mechanisms or several steps; full arrows indicate direct interactions. Activated TFEB induces many downstream cellular processes important for phagocyte function, including phagocytosis, lysosomes and autophagy, restoration of ER and mitochondrial homeostasis, lipid and glucose metabolism, and cytokine and chemokine production. Transcriptional mechanisms of *TFEB* regulation are not represented. GAL3, 8, galectins 3 and 8; TRIM16, tripartite motif containing 16; AMPK, AMP-activated protein kinase; FLCN, folliculin; TLR4, Toll-like receptor 4; TLR8, Toll-like receptor 8; LPS, lipopolysaccharide; AGS3, activator of G-protein signaling 3; AKT, v-Akt murine thymoma viral oncogene homolog; mTORC1, mechanistic target of rapamycin, complex 1; LAMTOR1, late endosomal/lysosomal adaptor, MAPK and mTOR activator 1; IFN- γ , interferon γ ; IFN γ R, INF- γ receptor; HIV, human immunodeficiency virus; TFEB, transcription factor EB; TGF- β , transforming growth factor β ; TGFBR, TGF- β receptor; ERK, extracellular signal regulated protein kinase; PC-PLC, phosphatidyl choline-directed phospholipase C; PrKD1, protein kinase D1; Fc γ R1, antibody Fc γ fragment receptor 1; HMOX1, heme oxidase 1; MCOLN1, mucolipin 1; ROS, reactive oxygen species; CO, carbon monoxide; Mtb, *M. tuberculosis*. This figure was created using BioRender (<https://biorender.com/>).

Key Figure, Figure 2. Direct and Indirect Regulation of Innate Immunity and Inflammation by TFEB in mammalian cells.

(a) Examples of direct mechanisms: TFEB and TFE3 bind directly to target gene promoters encoding cytokines IL-1 β and TNF- α , and SOCS3, a negative regulator of JAK/STAT signaling and pro-classical activation factor. (b) Examples of cellular processes and gene targets that mediate indirect regulation of innate immune cell function: TFEB and TFE3 promote autophagy and lysosomal expansion, lipid catabolism, and ER homeostasis. Collectively, these processes function to reduce inflammation in a cell-intrinsic manner. In addition, TFEB directly induces expression of PPAR- α and PGC-1 α , which promote mitochondrial biogenesis, inhibit NF- κ B, and reduce reactive oxygen species (ROS) production and inflammation. TFEB, transcription factor EB; IL-1 α , interleukin 1 α ; TNF- α , tumor necrosis factor α ; SOCS3, suppressor of cytokine signaling 3; JAK, Janus kinase; STAT, signal transducer and activator of transcription; PPAR- α , peroxisome proliferator activator receptor α ; PGC-1 α , PPAR- γ coactivator 1 α ; NF- κ B, nuclear factor κ B. This figure was created using BioRender (<https://biorender.com/>).

Table 1:

MiT Factors and Inflammatory Diseases

Disease	Function/observation	Mechanism	References
Atherosclerosis	Anti-inflammatory		
	Murine peritoneal macrophage TFEB activated by cholesterol crystals.	Cholesterol crystals damage lysosomes thus activating TFEB.	[34]
	Genetic and chemical induction of TFEB in macrophages protects mice from atherosclerosis.	Induction of autophagy and degradation of atherosclerotic plaques.	[34]
	Endothelial TFEB is necessary for VEGF signaling and proliferation of murine and human endothelial cells (EC).	TFEB was required for VEGF signaling in mice. TFEB functioned cell-autonomously in human and murine EC to promote EC proliferation <i>in vitro</i> and endothelial repair <i>in vivo</i> .	[68]
	Endothelial TFEB induces antioxidant genes and protects mice from vascular disease.	TFEB induces expression of HMOX1 and SOD1 in endothelium, reducing ROS and preventing NF- κ B induction and vascular disease in <i>db/db</i> mice.	[69]
Colitis	Anti-inflammatory		
	Deletion of <i>Tfeb</i> in the intestinal epithelium enhanced susceptibility of mice to chemically-induced colitis.	Mechanism unknown; suspected to be mediated by decreased TFEB-dependent expression of apolipoprotein A1.	[70]
Cystitis	Pro-inflammatory?		
	Activated by pro-inflammatory protease-activated receptors (PARs) in bladder epithelium of mice.	Unknown mechanism connecting PARs to TFEB	[55]
Hepatitis	Anti-inflammatory		
	Knockdown of <i>Tfeb</i> exacerbated ethanol-induced liver steatosis and injury in mice, while <i>Tfeb</i> overexpression ameliorated them.	Ethanol activated mTORC1, inhibiting TFEB and autophagy. Induction of autophagy increased β -oxidation of fatty acids and decreased triglycerides.	[71]
Pancreatitis	Anti-inflammatory		
	Deletion of <i>Tfeb</i> in pancreatic acinar cells enhanced cerulein-induced pancreatitis in mice; <i>Tfeb</i> <i>Tfe3</i> double knockouts developed spontaneous pancreatitis. Human pancreatitis biopsies showed decreased expression of TFEB.	Cerulein decreased autophagic flux and TFEB expression in mice; precise connection with inflammation is not well understood.	[72]