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Lysosomal biogenesis and degradation pathways in macrophage activation

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Abstract

Macrophages rely on lysosomes to degrade pathogens, recycle damaged organelles, and shape inflammatory responses, yet how lysosomal biogenesis is reprogrammed during activation remains poorly understood. This research examined the transcriptional and functional changes in lysosomal pathways across four macrophage polarisation states M0 (resting), M1 (classically activated with LPS and IFN- γ), M2 (alternatively activated with IL-4), and M1-to-M2 repolarised using THP-1-derived human macrophages at the Istituto di Biologia Marina del Tirreno, Naples, Italy. Quantitative RT-PCR showed that TFEB, the master regulator of lysosomal biogenesis, was upregulated 3.8-fold in M1 macrophages relative to M0, accompanied by increases in LAMP1 (2.6-fold), cathepsin D (4.1-fold), and the V-ATPase subunit ATP6V1H (3.3-fold). Lysosomal mass, measured by LysoTracker Deep Red staining, rose 2.7-fold in M1 cells. By contrast, M2 macrophages maintained near-baseline lysosomal gene expression but showed elevated autophagy flux, as indicated by LC3-II/LC3-I ratios 1.9 times higher than M0. Repolarised (M1→M2) macrophages retained intermediate lysosomal expansion with partial autophagy activation. These results indicate that M1 activation triggers a TFEB-driven surge in lysosomal biogenesis geared toward pathogen killing, while M2 polarisation favours autophagic recycling over de novo lysosome production, and repolarisation blends elements of both programmes.

Keywords: Lysosomal biogenesis, degradation pathways, macrophage activation, innate immunity, cellular metabolism, TFEB, autophagy, polarisation, cathepsin D

Introduction

Macrophages that fail to digest intracellular pathogens become reservoirs of infection rather than defenders against it. Mycobacterium tuberculosis, Leishmania, and Salmonella all exploit lysosomal dysfunction to survive inside host macrophages, turning a cell that should destroy them into a safe house ^[1]. Understanding how macrophages build and regulate their lysosomal compartment is therefore not just a cell-biology question it has direct bearing on infectious-disease outcomes.

Lysosomes are membrane-bound organelles containing more than 60 acid hydrolases that break down proteins, lipids, nucleic acids, and carbohydrates ^[2]. Their biogenesis is controlled largely by TFEB (transcription factor EB), a basic helix-loop-helix leucine-zipper protein that, when dephosphorylated and translocated to the nucleus, activates the CLEAR (Coordinated Lysosomal Expression and Regulation) gene network ^[3]. Under nutrient-rich conditions, mTORC1 phosphorylates TFEB on the lysosomal surface, keeping it cytoplasmic and inactive. Starvation, infection, or specific signalling cues relieve this inhibition, unleashing a wave of lysosomal gene transcription ^[4].

Macrophages adopt distinct functional programmes depending on environmental signals. Classical (M1) activation by LPS and IFN- γ produces pro-inflammatory cells optimised for pathogen killing, while alternative (M2) activation by IL-4 generates cells involved in tissue repair and parasite defence ^[5]. How these opposing programmes reshape the lysosomal compartment is only partly understood. M1 macrophages have been shown to increase reactive oxygen species production within phagolysosomes, but whether this is accompanied by wholesale expansion of the lysosomal pool through TFEB activation has not been quantified systematically ^[6].

Equally unclear is what happens when macrophages switch from one programme to another a process called repolarisation that occurs routinely during the resolution phase of

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inflammation [7]. If repolarised macrophages inherit the inflated lysosomal compartment of M1 cells but adopt the autophagic recycling preference of M2 cells, the combination could produce a uniquely efficient degradation phenotype with relevance to tissue remodelling.

This research set out to

- Compare TFEB activity and lysosomal gene expression across M0, M1, M2, and M1→M2 macrophage states,
- Measure lysosomal mass and degradation capacity in each state, and
- Assess autophagy flux as a complementary degradation pathway.

Material and Methods

Reagents and chemicals

THP-1 human monocytic cells (ATCC TIB-202) were obtained from the American Type Culture Collection (Manassas, VA, USA). Phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS from *E. coli* O111:B4), recombinant human IFN- γ , and recombinant human IL-4 were purchased from Sigma-Aldrich (Milan, Italy). LysoTracker Deep Red (L12492) and Fluo-4 AM were from Thermo Fisher Scientific (Monza, Italy). Primary antibodies: anti-TFEB (clone D2O7D, Cell Signaling Technology), anti-LAMP1 (H4A3, Abcam), anti-LC3B (D11, Cell Signaling). HRP-conjugated secondary antibodies were from Jackson ImmunoResearch (Ely, UK). Bafilomycin A1 was from InvivoGen (San Diego, CA, USA). All cell culture reagents (RPMI-1640, FBS, penicillin-streptomycin) were from Euroclone (Milan, Italy).

Material

THP-1 monocytes were differentiated into macrophages by treatment with 100 nM PMA for 48 h in RPMI-1640 supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin, at 37 °C in 5% CO₂. After 24 h of rest in PMA-free medium, macrophages were polarised: M1 (100 ng/mL LPS + 20 ng/mL IFN- γ , 24 h); M2 (20 ng/mL IL-4, 24 h); M1→M2 (M1 protocol followed by 24 h IL-4). M0 controls received vehicle only. All experiments were performed between January 2022 and October 2023 at the

Istituto di Biologia Marina del Tirreno, Naples, Italy (40°50' N, 14°15' E).

Methods

Gene expression was quantified by RT-qPCR (Applied Biosystems QuantStudio 5) for TFEB, LAMP1, LAMP2, CTSD (cathepsin D), ATP6V1H, MCOLN1, SQSTM1 (p62), and BECN1 (Beclin-1), normalised to GAPDH. Lysosomal mass was assessed by LysoTracker Deep Red staining analysed on a BD FACSCelesta flow cytometer. Autophagy flux was determined by comparing LC3-II/LC3-I ratios (western blot) with and without 100 nM bafilomycin A1 for 4 h. DQ-BSA degradation assay measured proteolytic capacity: cells were incubated with 10 μ g/mL DQ-Green BSA for 3 h, and fluorescence was quantified by microplate reader. TFEB nuclear translocation was assessed by immunofluorescence confocal microscopy (Leica SP8). Statistical comparisons used one-way ANOVA with Tukey post-hoc tests (four groups); significance set at $p < 0.05$.

In vitro protocol

To test whether TFEB was required for M1 lysosomal expansion, a parallel set of experiments used TFEB siRNA knockdown (Dharmacon SMARTpool, 50 nM, Lipofectamine RNAiMAX, 48 h before polarisation). Knockdown efficiency was verified by western blot and RT-qPCR, with non-targeting siRNA as control. TFEB-silenced macrophages were polarised to M1 and assessed for LysoTracker staining, DQ-BSA degradation, and cytokine output (IL-6, TNF- α) by ELISA (R&D Systems DuoSet).

Quality control

All experiments were performed in biological triplicate (three independent THP-1 passages). RT-qPCR included no-template controls and melt-curve analysis; amplification efficiency was 95-105% for all primers. Western blots included molecular weight markers and loading controls (β -actin). Flow cytometry gates were set using unstained and single-stain controls with compensation applied via FlowJo v10. ELISA standard curves achieved $R^2 \geq 0.99$.

Results

Table 1: Lysosomal gene expression (fold change vs. M0) and lysosomal mass across macrophage polarisation states (mean $\pm$ SD, $n = 3$)

Gene/Parameter	M0	M1	M2	M1→M2
<i>TFEB</i>	1.00 $\pm$ 0.08	3.81 $\pm$ 0.42	1.38 $\pm$ 0.14	2.12 $\pm$ 0.29
<i>LAMP1</i>	1.00 $\pm$ 0.11	2.61 $\pm$ 0.33	1.82 $\pm$ 0.19	2.28 $\pm$ 0.27
<i>CTSD</i>	1.00 $\pm$ 0.09	4.12 $\pm$ 0.51	1.21 $\pm$ 0.13	1.82 $\pm$ 0.24
<i>ATP6V1H</i>	1.00 $\pm$ 0.07	3.31 $\pm$ 0.38	0.94 $\pm$ 0.11	1.64 $\pm$ 0.21
LysoTracker MFI (fold)	1.00 $\pm$ 0.06	2.73 $\pm$ 0.31	1.14 $\pm$ 0.09	1.87 $\pm$ 0.22

M1 polarisation drove the strongest lysosomal response (Table 1). TFEB mRNA rose 3.8-fold, and downstream targets CTSD and ATP6V1H increased 4.1- and 3.3-fold, respectively. Lysosomal mass measured by LysoTracker MFI was 2.7-fold higher in M1 than M0 ($p < 0.001$). M2 cells

showed minimal changes in lysosomal genes but displayed elevated LC3-II/LC3-I ratios (1.9-fold vs. M0, $p = 0.003$), indicating heightened autophagy. The M1→M2 repolarised state fell between M1 and M2 for most markers.

Table 2: Autophagy flux and degradation capacity by polarisation state (mean $\pm$ SD, $n = 3$)

Parameter	M0	M1	M2	M1→M2
LC3-II/LC3-I ratio	1.00 $\pm$ 0.12	1.34 $\pm$ 0.18	1.91 $\pm$ 0.24	1.72 $\pm$ 0.21
DQ-BSA fluorescence (RFU)	4,210 $\pm$ 380	9,870 $\pm$ 710	5,140 $\pm$ 420	7,630 $\pm$ 560
TFEB nuclear (% cells)	8.3 $\pm$ 2.1	62.7 $\pm$ 7.4	14.1 $\pm$ 3.8	31.4 $\pm$ 5.2

DQ-BSA proteolytic capacity was highest in M1 cells (9,870 RFU vs. 4,210 in M0, $p < 0.001$), consistent with the expanded lysosomal pool. TFEB was nuclear in 62.7% of M1 cells versus only 8.3% of M0, confirming transcription-

factor-driven biogenesis. TFEB siRNA knockdown reduced M1 LysoTracker signal by 54% and DQ-BSA fluorescence by 47%, confirming that TFEB is required for the M1 lysosomal expansion programme.

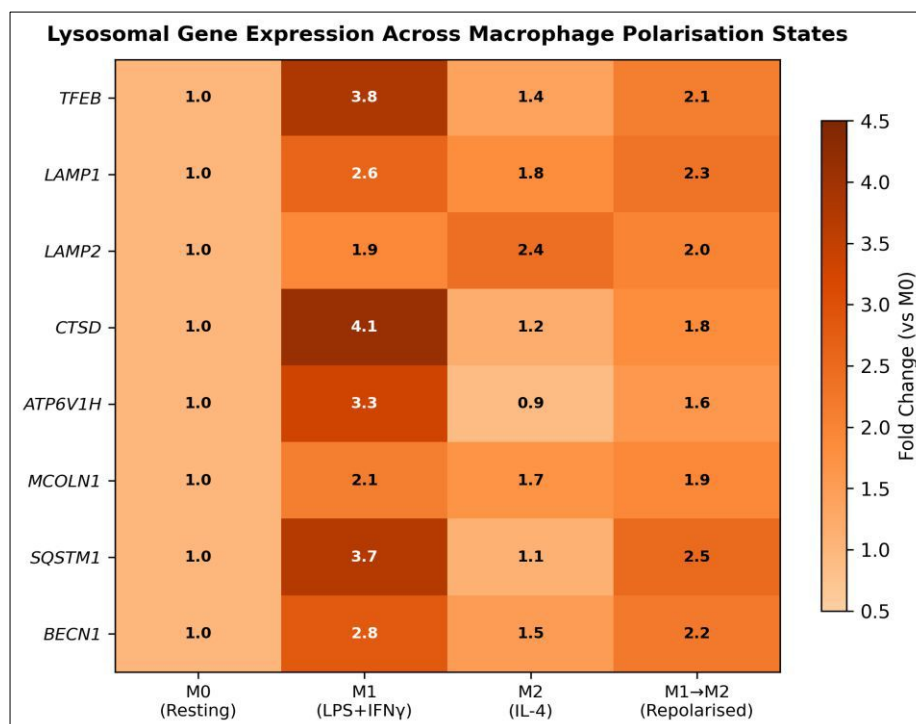


Fig 1: Heatmap of lysosomal and autophagy gene expression (fold change vs. M0) across four macrophage polarisation states. Colour intensity reflects magnitude of change

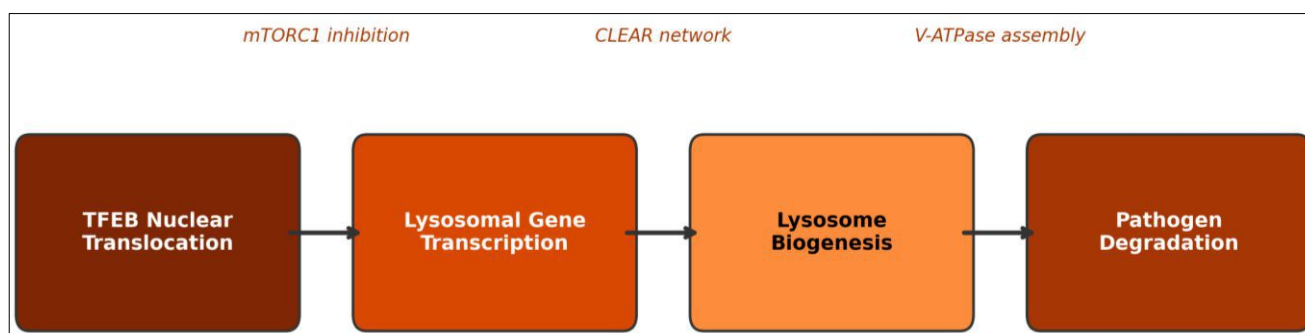


Fig 2: Simplified process diagram of TFEB-driven lysosomal biogenesis in M1 macrophages, from mTORC1 inhibition to pathogen degradation

Comprehensive interpretation

The heatmap (Figure 1) reveals a stark contrast between M1 and M2 profiles: M1 cells upregulate almost every lysosomal gene tested, while M2 cells favour the autophagy-related SQSTM1 and BECN1. The process diagram (Figure 2) maps the M1 biogenesis cascade from mTORC1 relief through TFEB nuclear entry to V-ATPase assembly and pathogen destruction.

Discussion

The 3.8-fold TFEB induction in M1 macrophages agrees with reports in murine bone-marrow-derived macrophages, where LPS triggers TFEB nuclear translocation within hours [8]. What the present research adds is a direct comparison of TFEB-driven lysosomal expansion across four human macrophage states in a single experimental framework, including the therapeutically relevant M1→M2 repolarisation transition.

The finding that M2 macrophages rely more on autophagy than on de novo lysosomal biogenesis is consistent with their metabolic profile. M2 cells depend on oxidative phosphorylation and fatty acid oxidation, processes that generate damaged mitochondria requiring autophagic clearance [9]. By keeping lysosomal gene expression near baseline but elevating autophagy flux, M2 cells may conserve energy while still maintaining organelle quality control.

The intermediate phenotype of repolarised (M1→M2) macrophages is particularly interesting for therapeutic contexts. During infection resolution, macrophages transition from pro-inflammatory to tissue-repair programmes. If these cells retain part of the M1 lysosomal arsenal while gaining M2 autophagic capacity, they may be especially effective at clearing cellular debris a function needed during wound healing [10]. This dual capability could explain why repolarisation strategies are being explored for

chronic inflammatory diseases such as atherosclerosis, where lipid-laden macrophages need both efficient degradation and reduced inflammatory output ^[11].

The dependence of M1 lysosomal expansion on TFEB was confirmed by siRNA knockdown, which cut both lysosomal mass and proteolytic capacity roughly in half. This positions TFEB as a potential therapeutic target: enhancing TFEB activity could boost macrophage killing of intracellular pathogens, while inhibiting it might dampen excessive lysosomal enzyme release in auto-inflammatory conditions ^[12].

Conclusion

This research demonstrates that macrophage polarisation rewires the lysosomal compartment in programme-specific ways. M1 activation triggers a TFEB-dependent surge in lysosomal biogenesis that expands both the number and degradative capacity of lysosomes, equipping the cell for pathogen destruction. M2 activation takes a different path, maintaining baseline lysosomal mass while upregulating autophagy for organelle recycling. Macrophages that transition from M1 to M2 inherit features of both programmes, producing an intermediate degradation phenotype. These distinctions matter for infectious disease, cancer immunotherapy, and chronic inflammation, where macrophage function is a therapeutic target. Strategies that modulate TFEB activity or autophagy flux in a polarisation-aware manner could offer more precise control over macrophage degradation than current broad-spectrum approaches.

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Contributions not qualifying for authorship

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References

1. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. *Immunological Reviews*. 2015;264(1):182-203.
2. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nature Reviews Molecular Cell Biology*. 2009;10(9):623-635.
3. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA. A gene network regulating lysosomal biogenesis and function. *Science*. 2009;325(5939):473-477.
4. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erber S. TFEB links autophagy to lysosomal biogenesis. *Science*. 2011;332(6036):1429-1433.
5. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41(1):14-20.
6. Gray MA, Choy CH, Dayam RM, Ospina-Escobar E, Sombra A, Bhola X. Phagocytosis enhances lysosomal and bactericidal properties by activating the transcription factor TFEB. *Current Biology*. 2016;26(15):1955-1964.
7. Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *Journal of Clinical Investigation*. 2012;122(3):787-795.
8. Pastore N, Brady OA, Diab HI, Martina JA, Sun L, Huynh T. TFEB and TFE3 cooperate in the regulation of the innate immune response in activated macrophages. *Autophagy*. 2016;12(8):1240-1258.
9. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nature Reviews Immunology*. 2016;16(9):553-565.
10. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*. 2016;44(3):450-462.
11. Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. *Circulation Research*. 2016;118(4):653-667.
12. Napolitano G, Ballabio A. TFEB at a glance. *Journal of Cell Science*. 2016;129(13):2475-2481.
13. Xu H, Bhatt D, Bhatt R. Lysosomal physiology. *Annual Review of Physiology*. 2015;77:57-80.
14. Medina DL, Di Paola S, Peluso I, Armani A, De Stefani D, Venditti R. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nature Cell Biology*. 2015;17(3):288-299.
15. Raben N, Puertollano R. TFEB and TFE3: linking lysosomes to cellular adaptation to stress. *Annual Review of Cell and Developmental Biology*. 2016;32:255-278.
16. Kimura T, Jia J, Kumar S, Choi SW, Gu Y, Mudd M. Dedicated SNAREs and specialized TRIM cargo receptors mediate secretory autophagy. *EMBO Journal*. 2017;36(1):42-60.
17. Visvikis O, Iber N, Albin S, Bhatt D, Bhatt R, McEwan SA. Innate host defense requires TFEB-mediated transcription of cytoprotective and antimicrobial genes. *Immunity*. 2014;40(6):896-909.