Bacterial pathogenesis

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Autophagy is part of the answer to tuberculosis

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Almost twenty years after it was first linked to control of *Mycobacterium tuberculosis* in macrophages, autophagy retakes centre stage, as shown in murine models and human cells.

Mycobacterium tuberculosis infections are estimated to have caused 1.6 million deaths worldwide in 2022. According to the World Health Organization, deaths from tuberculosis (TB) have increased, despite under-reporting of new TB cases owing to COVID-19. Research with *M. tuberculosis* is notoriously laborious, because of slow replication of the tuberculous bacilli and a lengthy disease course, sometimes up to 200 days when modelled in mice. In 2004, autophagy was reported to be part of the macrophage innate immune defence against *M. tuberculosis* infection[1]. This finding was initially validated in mouse models in 2012 (refs. 2,3), but a substantive role for autophagy in defence against *M. tuberculosis* was largely ruled out by an influential study that was published in 2015 (ref. 4). Now reporting in *Nature Microbiology*, two studies revisit the role of canonical autophagy, and related non-canonical processes, and find that autophagy confers protection against *M. tuberculosis* infection in human and murine cells, and in mice[5,6]. These findings will rekindle interest in the potential of autophagy as a therapeutic target for TB control, almost twenty years after the first report linking autophagy to host defence against this most devastating infectious disease.

Canonical autophagy is a homeostatic process that is central to human health; it cleanses cellular cytoplasm by breaking down and removing waste macromolecules and organelles, and kills invading microorganisms[7]. Autophagy has an important role in host defence and immunity. In principle, autophagy also suppresses inflammation, whereas excessive inflammation can cause disease pathology[7]. In 2004, the first study showing that induction of autophagy restricts virulent *M. tuberculosis* in macrophages (its target cell) from mice and humans was published[1].

Later, in vivo studies using murine models of TB, in which *Atg5* (*Atg5*$^{fl/fl}$ *LysMcre*) was conditionally inactivated in myeloid cells, reported high mortality and excessive inflammation[2,3]. *Atg5* is one of the core autophagy genes (Fig. 1). These mouse studies used low-dose aerosol exposure that causes a chronic disease, and models the typical presentation of human TB[2,3]. A subsequent analysis[4] used conditional knockouts in multiple autophagy genes, first confirming that *Atg5*$^{fl/fl}$ *LysMcre* mice were particularly susceptible to *M. tuberculosis* and then establishing that neutrophilic inflammation accounted for pathology and mortality[4]. However, in the same study, mice with conditional knockouts in several other autophagy genes, *Atg14*, *Ulk1/2* (*Atg1*), and the enzymatic components *Atg3*, *Atg7*, *Atg12*, and *Atg16L1* carrying out LC3 lipidation (also referred to as membrane atg8ylation[7]; Fig. 1) did not succumb to *M. tuberculosis* infection within 80 days post-infection[4]. These findings led to general acceptance that autophagy might not be a useful target for therapeutics for *M. tuberculosis*, as reflected in the accompanying News and views article, which was titled 'Autophagy is not the answer'[8].

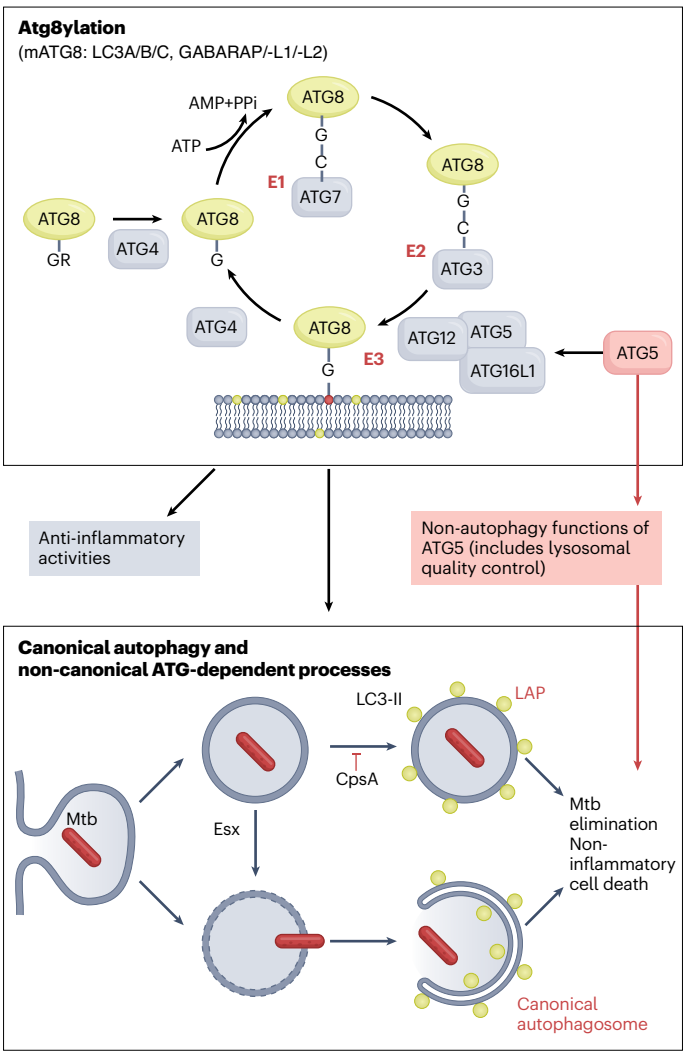

Fig. 1 | Canonical and non-canonical ATG-dependent processes contribute to control of *M. tuberculosis*. Membrane atg8ylation with mATG8 proteins (top box) leads to anti-inflammatory and antimicrobial effects including canonical and non-canonical processes (bottom box) such as LAP and canonical autophagy. Prior studies and recent articles including two in this issue of *Nature Microbiology* re-establish the role of canonical autophagy and non-canonical atg8ylation-dependent processes in control of *M. tuberculosis* (Mtb) both in vivo and ex vivo. E1, E2 and E3, ligases in the atg8ylation cascade analogous to the E1, E2 and E3 ligases in the ubiquitylation cascade; LC3A/B/C, mATG8 proteins LC3A, LC3B and LC3C; GABARAP/-L1/-L2, mATG8 proteins GABARAP, GABARAPL1 and GABARAPL2.

In the first of two studies published now in *Nature Microbiology*, Golovkine et al.[5] considered the possibility that conditional inactivation of floxed *Atg* genes with the *LysMcre* driver might have been incomplete

for certain genes. The authors painstakingly titrated *Cre*-driven *loxP*-excision events, by increasing *Cre* recombinase gene dosage in mice bred to be homozygous for *LysMcre* (*LysMcre*$^{+/+}$). This approach enabled better control of gene expression than in mice carrying only one *LysMcre* allele (*LysMcre*$^{+/-}$), which was previously considered to be sufficient for *Cre*-driven *lox*-excision. Their strategy enabled more complete inactivation of *Atg7* and *Atg16L1* genes, and resulted in increased susceptibility of *LysMcre*$^{+/+}$ *Atg7*$^{fl/fl}$ and *LysMcre*$^{+/+}$ *Atg16L1*$^{fl/fl}$ mice to *M. tuberculosis* compared to *Cre*$^{-}$ littermates. In parallel, *LysMcre*$^{+/-}$ *Atg7*$^{fl/fl}$ mice showed no increased susceptibility to infection, but *LysMcre*$^{+/-}$ *Atg16L1*$^{fl/fl}$ mice carrying only one *LysMcre* allele displayed increased susceptibility to *M. tuberculosis*, which was probably missed in the previous study[4] owing to shorter duration of observations. Additional experiments by Golovkine et al. in *LysMcre*$^{+/+}$ *Atg7*$^{fl/fl}$ and *LysMcre*$^{+/+}$ *Atg16L1*$^{fl/fl}$ mice confirmed all prior observations regarding increased lesions and neutrophilic infiltration reported for *Atg5*$^{fl/fl}$ *LysMcre* mice[2–4] and the authors observed dynamic changes in bacterial burden. Thus, this study proves that multiple autophagy genes are needed for disease control in the mouse model of *M. tuberculosis* infection.

The study by Golovkine et al. reaffirmed the distinct phenotype of *Atg5* relative to other atg8ylation genes: *M. tuberculosis*-infected mice depleted for Atg5 in myeloid cells died faster than the properly depleted Atg7 or Atg16L1 mice. The special role of Atg5 in vivo was also identified in a recent study by Wang et al.[9]. Mechanistically, Atg5 has 'moonlighting' jobs in addition to its function in canonical autophagy. The study by Wang et al.[9] indicates that Atg5 helps to maintain functional lysosomes and affects exocytosis. In the absence of Atg5, instead of the canonical Atg12–Atg5 conjugate (Fig. 1), Atg12 engages in the formation of the non-canonical Atg12–Atg3 conjugate that binds ALIX thus redirecting this ESCRT protein from lysosomal repair tasks to exocytic events[9]. In neutrophils, this causes excessive activation and degranulation[9], explaining in part the unique standing of Atg5 relative to other components of the atg8ylation machinery.

Both Golovkine et al. and Wang et al.[9] reaffirmed a role for autophagy in *M. tuberculosis* control, but it still remained unclear what the contribution of non-canonical processes were. Of note, the findings of Golovkine et al. were focused on Atg7 and Atg16L1. These Atgs are specific for the atg8ylation process[7] that conjugates mammalian Atg8s to membrane phospholipid phosphatidylethanolamine, commonly referred to as LC3 lipidation. Atg8ylation is not restricted to canonical autophagy (Fig. 1) and participates in a variety of membrane stress and remodelling responses, the extent of which is only beginning to be unravelled, encompassing LC3-associated phagocytosis (LAP) and a variety of other processes[7].

LAP has previously been implicated in control of *M. tuberculosis* using bacilli lacking CpsA, which indirectly inhibits LAP via NOX2 but only during innate responses, as interferon-γ and adaptive immunity abrogate CpsA effects both ex vivo and in vivo[10]. Interferon-γ induces autophagy[1]. Also now reporting their findings in *Nature Microbiology*, Aylan et al.[6] aimed to assess the relative contributions of LAP and canonical autophagy in control of *M. tuberculosis* by comparing *M. tuberculosis* Esx and CpsA mutants. The ESX-1 Type 7 secretion system is important for *M. tuberculosis* escape from the phagosome[2] whereas CpsA inhibits LAP[10]. Adding to the complexity of the experimental design, CpsA binds canonical autophagy cargo receptors NDP52 and TAX1BP[10]. It is also not clear how CpsA enters the host cytosol, and this might depend on permeabilization by Esx. Aylan et al. infected human induced pluripotent stem cell-derived macrophages lacking ATG14 (considered to be specific for canonical autophagy) with *M. tuberculosis* and compared the outcomes with infection of ATG7 (affects both autophagy and LAP) with *M. tuberculosis*. The results were a combination of the expected and the unexpected. As one might anticipate, based on the multifaceted outputs of atg8ylation (Fig. 1), the loss of ATG7 permitted growth of all strains tested (wild-type *M. tuberculosis* and CpsA and Esx mutants) thus not discerning between canonical autophagy and non-canonical effects of atg8ylation. Also, Golovkine et al. observed that murine macrophages defective in atg8ylation, that is, mutants in Atg5, Atg7 and Atg16L1, could not limit Esx-mediated phagosome disruption or cytosolic access by mycobacteria. One consequence of this was that cell death of atg8ylation-deficient macrophages infected with *M. tuberculosis* switched from less-inflammatory apoptosis to a more pro-inflammatory necrotic type of cell death[5], reminiscent of previously reported observations[3]. The findings of Golovkine et al. were corroborated by Aylan et al. in human induced pluripotent stem cell-derived macrophages lacking ATG7. Further surprises came when Aylan et al. also reported that loss of *ATG14* (considered to be a canonical autophagy gene) restricted a *M. tuberculosis esx* mutant, which was not anticipated to be subject to canonical autophagy as it would be confined to the phagosome[6]. This led Aylan et al. to propose that ATG14 affects phagosomal maturation.

The field of autophagy has matured and grown[7] since the initial report of its effects on *M. tuberculosis* infection[1]. Canonical autophagy is a part of a membrane stress and remodelling response (including LAP) that is controlled by the atg8ylation machinery[7]. The findings of Golovkine et al. highlight the in vivo role of atg8ylation components in canonical autophagy. The findings of Aylan et al. and Wang et al.[9] highlight roles for both canonical and non-canonical processes in *M. tuberculosis* control.

While the path to autophagy-based interventions for TB might be complex, these studies will rekindle interest in the potential for autophagy-directed therapies. Pharmacological targets need to be redefined as we continue to learn about the role of autophagy in host–pathogen interactions.

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Competing interests

The authors declare no competing interests.