Indian J Microbiol (Oct–Dec 2015) 55(4):456–459
DOI 10.1007/s12088-015-0541-9

OPINION ARTICLE

The Tussle Between Mycobacteria and Host: To Eat or Not To Eat

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Received: 26 May 2015 / Accepted: 4 July 2015 / Published online: 11 July 2015

Abstract Autophagy is a catabolic process of cellular homeostasis evolutionarily conserved in eukaryotes. To block infection of intracellular bacterial pathogens, metazoans deploy autophagy for pathogen clearance through phago-lysosome formation and specific bactericidal peptides. Although an array of research have publicized the host regulatory factors, the function of bacterial effectors are yet to be understood in detail. In this article, we focus on the autophagic response to one of the most successful intracellular bacteria *Mycobacterium tuberculosis*.

Keywords Tuberculosis · Autophagy · Host–pathogen interaction · Virulence factors · Mycobacteria · *Mycobacterium tuberculosis*

Mycobacterium tuberculosis, the causative agent of tuberculosis, has probably killed more people than any other microbial pathogen in the history of human civilization. The emerging drug-resistant forms of *M. tuberculosis* coupled with HIV co-infection pose a threat never felt before. Although various reasons have been suggested for development of drug resistance among different microbes [1], the ways with which *M. tuberculosis* adapt and improvise in order to survive remains a mystery. Apart from possessing a plethora of regulatory proteins for its survival and pathogenicity [2, 3], it is also known to secrete a gamut of virulence factors to thwart of host immune-defence [4]. This scenario warrants investigation both towards search of new drug targets as well as discovery of new drugs [5–7]. Fortunately in the recent years several drugs have been developed to address the issue of multi-drug resistance tuberculosis, two prominent examples being TMC-207 (bedaquiline) which is already granted approval and 24-desmethylrifampicin (a rifamycin derivative), a drug found to be effective against multidrug-resistance tuberculosis [8, 9]. Although studies are underway to see the effect of newer candidates against mycobacterial infections, we are ignorant about the effect of anti-mycobacterial antibiotics on host–pathogen tussle especially in autophagy networks. One study indicates that azithromycin blocks autophagy pathway and its long term use predispose cystic fibrosis patients to non-tuberculous mycobacterial infections [10]. However, the mycobacterial infection process comprises of a complicated patho-physiology which needs to be thoroughly understood before therapeutic intervention.

Autophagy is a protective cellular process ubiquitously present in eukaryotic organisms. Although the facets of autophagy have been known for quite some time, recent advances in the past decade have thrown new light on autophagy as an immune-defence mechanism against tuberculosis infection. It is now perceived that host deploy autophagy to tackle *M. tuberculosis* in two different ways: one to eradicate mycobacteria by promoting the autophagosome–lysosome fusion and the other to deliver mycobactericidal peptides/enzymes in the mature lysosomal vesicles [11]. However, the ability of *M. tuberculosis* to utilize its virulence factors to avoid the phago-lysosomal fusion and establish the infection is well documented [4]. Despite decades of ongoing research on various aspects of tuberculosis, we are still data deficient on the exact

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mechanism of granuloma formation and transmission from the perspective of the involved mycobacterial factors. To add to the complexity, it is now emerging that different strains or lineages of *M. tuberculosis* could behave differently in infection process and even within a single host there could be different strategies employed by mycobacteria. Perhaps that's why it is not surprising to know that mycobacteria could escape to cytosol in contrast to the classical view of capturing the pathogen in phagosome compartment [12]. The ability of mycobacteria to have a back-up route for all the defence manoeuvres it encounters is a fact that is hard to fathom. Autophagy is no exception.

An extensive array of research has revealed the intricate network of macroautophagy in tuberculosis [11]. However, most of it is directed towards the host factors and we still understand very little about the contribution or counteraction of mycobacterial virulence factors. Murine LRG47, orthologue of human IRGM (immunity-related GTPase family, M) was the first of such host factors to be identified which promotes autophagy in a process involving interferon gamma (IFN-γ) a key pro-inflammatory cytokine [13, 14]. Several cytokines have displayed mixed profile in mycobacterial infection and subsequent autophagy processes. While Th1 response seems to favour autophagy, cytokines from the Th2 groups have been shown to be counteractive [15]. Recent reports also speculate function of IL-1 and Th17 responses in bacterial containment and regulation of inflation [11]. Very recently, a cyclic dinucleotide has also been reported to induce autophagy via IFN-β induction [16]. Another group of fine-tuning regulators are the microRNAs which regulate gene expression of many immunity related genes by targeting mRNAs for translational repression or degradation [17]. Recent trends suggest modulation of autophagy by these regulatory RNAs; however, the exact role remains unclear. While miR-155 is found to be involved in autophagy through inhibition of Ras homologue enriched in brain (RHEB) [18], two other microRNAs, namely miR-125a and miR-30A, have been shown to abrogate autophagy [19, 20]. The other dimension of autophagy research involves bactericidal proteins/enzymes exploited by host. Ubiquitylated-peptides and p62 are some of the host factors with proven action against mycobacteria [21, 22]. While a number of cellular factors have been reported, the mycobacterial counter-response to autophagy is a complex and less-understood scenario (Table 1). Two proteins, Eis and LpqH are reported to be involved in contrasting manner. While LpqH is involved in autophagy regulation through calcitriol (1,25-dihydroxyvitamin D3) mediated vitamin D receptor signalling, the Eis (enhanced intracellular survival) protein inhibits autophagy with a process thought to involve dual specificity protein phosphatase 16 and redox signalling [23, 24]. Our work with secreted modulatory protein PpiA has revealed a number of interacting partners in host which are directly or indirectly linked with various autophagy pathways. These include the vacuolar ATPase subunit ATP6VOE1, the well-documented autophagy protein STING/TMEM173 (Stimulator of interferon genes), and KXD1 (KxDL motif containing 1) [25]. There is an urgent need to identify other such mycobacterial factors aimed at influencing/modulating autophagy to unveil mycobacterial survival strategies. One such intricate phenomenon involves the type seven secretion system (T7SS or ESX) of mycobacteria. ESX systems are known for secreting virulence factors but a recent study highlighted the involvement of ESX-1 in promoting autophagy through permeabilization of phagosomal membrane due to which extracellular DNA is detected by autophagy regulator STING [26]. However, it could be a ploy by mycobacteria to increase the pro-inflammatory response to create a habitable micro-niche/granuloma for itself. The support for this argument comes from another study where ESX system is shown to inhibit autophagy in dendritic cells [27]. While we were writing this article, three studies independently and simultaneously identified cyclic GMP–AMP synthase (cGAS) as the host sensor for mycobacterial extracellular DNA. The collective understanding from these studies reveals that the activation of IFN-β is mediated through STING/TBK1/IRF3 signal transduction which is initiated through cGAMP produced by cGAS upon detection of mycobacterial DNA [28–30]. On the pathogenic front, a previously known virulence factor is also recently shown to be involved in autophagy. Work by Hu and colleagues suggest that mycobacterial acid phosphatase SapM targets host Rab7 GTPase to inhibit the fusion of autophagosome and lysosome [31]. The autophagic response against mycobacteria is a multipartite process involving pathogen recognition, phagosome maturation and lysosomal targeting of mycobactericidal peptides, the complexity of which has only started to unfold [32–34].

While host-autophagy modulating agents and cellular autophagy processes are being studied extensively, several questions need to be addressed in the mycobacterial front. The foremost need is to identify all the mycobacterial factors involved in inhibiting/modulating the autophagy processes. Do mycobacteria have designated virulence factors to tackle autophagy? Do the active and latent mycobacteria behave differently in tackling autophagy? What happens to the bacteria that escape to cytosol? Investigating these pathogen perspectives will give a better understanding of the temporal and spatial regulation of autophagy inside the host. The observations that there are both pro and anti-autophagic factors inside mycobacteria escalate the complexity of mycobacterial population dynamics. If mycobacterial population deliberately

Table 1 Regulatory factors involved in promoting/inhibiting autophagy

Regulatory factors	Autophagy	Interacting protein partners/target genes	References
Host			
IRGM, IFN-γ	Promotes	IFN-γ/starvation induced autophagy: Beclin1/ATG7	[14, 34]
miR-155	Promotes	Ras homologue enriched in brain (RHEB)	[18]
SQSTM1/p62	Promotes (bactericidal)	Ribosomal protein S30 (RPS30/FAU)	[21]
Ub-peptides	Promotes (bactericidal)	Inner mycobacterial membrane	[22]
IL-1β	Promotes	TANK-binding kinase 1 (TBK1)	[32]
DRAM1	Promotes	STING/TMEM173, Sequestosome-1 (SQSTM1)	[33]
cGAS	Promotes	STING, TBK1, IRF3	[28–30]
IL-4, IL-13	Inhibits	Akt/PKB signaling pathway	[15]
miR-30a	Inhibits	Negative correlation with Beclin-1 (BECN1/ATG6)	[19]
miR-125a	Inhibits	UV radiation resistance associated gene (UVRAG)	[20]
Mycobacterial			
disA/dacA	Promotes	Type I IFN response via IFN-β induction	[16]
LpqH	Promotes	Vitamin D receptor signaling, cathelicidin (CAP18/LL-37)	[24]
ESX1/eDNA	Promotes	Stimulator of interferon genes (STING/TMEM173)	[26]
PpiA	Modulatory	ATP6VOE1, STING/TMEM173, KXD1	[25]
Eis	Inhibits	Dual specificity protein phosphatase 16	[23]
ESX1	Inhibits	–	[27]
SapM	Inhibits	Rab7	[31]

sacrifices few co-inhabitants in order to jump-start the inflammation process, it remains to be seen whether the therapeutic strategies enhancing autophagy will actually be effective or counter-productive.

Several aspects of autophagy during the course of tuberculosis infection process are being investigated by scientists around the world and the list is ever-increasing. A thorough understanding of autophagosome-mycobacterial interaction with equal emphasis to both the partners could give us better leads to pharmacological intervention strategies.

Acknowledgments We are thankful to Dr. Niti Pathak and Dr. Mahesh Dhar for critical reading of the manuscript. We are thankful to Director, Cluster Innovation Centre for providing the platform to carry our research.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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