

REVIEW ARTICLE

Comparison of immune evasion mechanisms of multidrug-resistant *Mycobacterium tuberculosis* versus nontuberculous mycobacteria

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Abstract

Mycobacterium tuberculosis (MTB) and nontuberculous mycobacteria (NTM) are important worldwide health problems because they may challenge human immunity and cause chronic infections. This study presents a comparative overview of immune evasion mechanisms in multidrug-resistant MTB (MDR-MTB) and NTM, focusing on mechanisms supported by direct experimental evidence and those inferred from drug-susceptible MTB. Supported findings in MDR-MTB include LAM-mediated block of phagolysosomal fusion, depletion of PI3P by SapM, and Zmp1-mediated suppression of AIM2 inflammasome activation. Mutations in *katG* and *rpoB* confer resistance to isoniazid and rifampicin. However, these primarily affect drug susceptibility rather than immune modulation. Other processes, such as ESX-1-mediated membrane disruption, PPE protein interference with oxidative stress, and ubiquitination pathway evasion, are well described in drug-susceptible MTB and are inferred to contribute to MDR-MTB persistence. For NTM, experimental evidence demonstrates immune evasion through biofilm formation (glycopeptidolipids, PKS1, polyphosphate accumulation), active efflux pumps, and cytokine modulation via LAM and Toll-like receptor 2 (TLR2) blockade. NTM also inhibits phagolysosomal fusion using SapM and PtpA, while granuloma formation sustains long-term infection. Both bacteria use overlapping mechanisms, including inhibition of phagosome-lysosome fusion, modulation of cytokine responses, and granuloma formation, but differ in their ecological niches, transmission, and clinical presentations. These immune-evasive features complicate therapy, prolong treatment, and hamper vaccine development. Emerging therapeutics include autophagy inducers (e.g., rapamycin) and host-directed therapies (e.g., interferon-gamma). Future prospects include personalized medicine, innovative vaccination platforms such as messenger RNA-based techniques, and a One Health approach to address the interconnected issues of MDR-MTB and NTM. Thus, this review explicitly distinguishes experimentally validated immune evasion mechanisms underlying MDR-MTB from those extrapolated from drug-susceptible strains, while integrating evidence-based findings for NTM.

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doi: 10.36922/M1026210052**Received:** May 22, 2026**Revised:** June 22, 2026**Accepted:** July 1, 2026**Published online:** July 22, 2026**Copyright:** © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.**Publisher's Note:** AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.**Keywords:** Multidrug-resistant *Mycobacterium tuberculosis*; Nontuberculous mycobacteria; Immune evasion mechanisms; Phagosome-lysosome fusion inhibition; Biofilm persistence; Host-directed therapies

1. Introduction

Tuberculosis (TB) is one of the most common respiratory tract infections caused by the notorious *Mycobacterium tuberculosis* (MTB); according to the World Health Organization (WHO), approximately 10.6 million new cases and 1.3 million TB deaths were reported worldwide in 2022.¹ TB is one of the most ancient diseases, dating back to medieval times, and is also known as the “white plague.”² The most common symptoms are shortness of breath, night sweats, weight loss, and lung pain.³ TB is a major opportunistic infection in HIV-positive individuals.⁴

Mycobacterium tuberculosis is a rod-shaped bacillus surrounded by a thick lipid layer and mycolic acid, which protect it from host immune recognition. It contains approximately 4.41 million base pairs, with a high G+C content (~80%), and is rich in Pro-Glu and Pro-Pro-Glu (PPE) motifs.⁵ The genome of MTB consists of 50 RNA genes and 3,924 protein-coding genes.⁶ It belongs to the family Mycobacteriaceae. Due to incomplete antibiotic regimens in infected patients, genetic resistance has developed in MTB, leading to the emergence of multidrug-resistant MTB (MDR-MTB), which is becoming increasingly difficult to treat as first-line antibiotics become ineffective.^{7–9}

Meanwhile, another type of bacteria, similar to MTB, has emerged: nontuberculous mycobacteria (NTM). NTM mimics MTB during infection, leading to misdiagnosis and inappropriate treatment.¹⁰ Unlike MTB, NTM are not transmitted from person to person; they are usually transmitted through environmental sources, such as water and soil.¹¹ They primarily affect the elderly and those with pre-existing or weakened respiratory problems.¹²

Both MTB and NTM can evade the human immune system, posing a significant global health problem, particularly amid increasing antibiotic resistance. They have a range of mechanisms to evade the immune system, allowing them to thrive and persist within the host and defeat the host's antimicrobial defenses, leading to a persistent infection. This increases the risk of pathogen survival and leads to therapy failure. Thus, it is vital to understand the mechanisms of immune evasion to develop effective treatment strategies.

However, comparative outcomes between MDR-MTB and NTM are lacking, and this is a crucial research need. This review is new in that it systematically compares MDR-MTB and NTM, separating empirically confirmed processes in MDR-MTB from those derived from drug-susceptible strains, and merging them with evidence-based NTM investigations. It is not limited to descriptive summaries and highlights translational implications

and future directions, making a unique contribution to the mycobacterial literature by linking immune evasion strategies to therapeutic barriers, vaccine development hurdles, and host-directed interventions. This review presents a direct comparison framework for MDR-TB and NTM, which, unlike earlier studies that generally discussed immune evasion only in drug-susceptible MTB or NTM alone, fills a gap in the literature.^{13–15}

This review examines the immune evasion mechanisms of MDR-TB and NTM, focusing on their molecular interactions with the host immune system.¹⁶ It investigates major processes including suppression of phagolysosomal fusion, granuloma development, biofilm production, alteration of cytokine responses, and escape from the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) signaling pathway.^{17–19} In addition, the review evaluates the effect of these immune evasion mechanisms on clinical care, vaccine development, and treatment methods. It also focuses on identifying potential targets, such as autophagy activators and host-directed medicines, that may overcome therapeutic hurdles and improve patient outcomes.^{20,21}

2. Overview of mycobacterial pathogens

2.1. *Mycobacterium tuberculosis*

Multidrug-resistant TB refers to infection with strains of MTB that are resistant to isoniazid and rifampicin, the two most powerful anti-TB drugs.⁷ MDR-TB is a major global health threat, responsible for approximately 410,000 new cases in 2022 (or 3.5% of all TB cases) and primarily affecting regions with weak healthcare infrastructure, including Eastern Europe, Asia, and sub-Saharan Africa.^{22,23} The emergence of extensively drug-resistant TB (XDR-TB), resistant to both first- and second-line therapies, further underscores an urgent need for innovative solutions.^{24,25}

Multidrug-resistant MTB retains the characteristic properties of being acid-fast, replicating slowly, and surviving inside cells. It acquires resistance primarily through mutations in genes such as *katG* (resistant to isoniazid) and *rpoB* (resistant to rifampicin).^{8,9}

However, these resistance-conferring mutations primarily alter drug-target interactions rather than directly modulating host immune pathways. Current evidence indicates that immune evasion in MDR-TB is largely mediated by virulence factors (e.g., secreted acid phosphatase M [SapM], PPE proteins, early secretory antigenic target 6 kDa [ESAT-6], zinc metalloprotease 1 [Zmp1]) rather than *katG* or *rpoB* mutations themselves.^{26,27} Thus, drug resistance and immune evasion represent parallel but distinct survival strategies, although

prolonged persistence due to resistance indirectly enhances opportunities for immune evasion.

Multidrug-resistant MTB has a lipid-rich cell wall, rich in mycolic acids, which protects it from antibiotics and host defenses.^{28,29} Proteins such as protein kinase E (PknE) help inhibit the fusion of phagosomes with lysosomes within macrophages.¹⁶ Enzymatic defenses against oxidative stress and secreted virulence factors, such as ESAT-6 and culture filtrate protein 10 kDa (CFP-10), also facilitate immune evasion and chronic persistence.^{30,31} These characteristics mean MDR-MTB is a difficult and resistant pathogen to treat.³²

2.2. Nontuberculous mycobacteria

Nontuberculous mycobacteria, also known as atypical mycobacteria, are a diverse group of species other than the MTB complex and *Mycobacterium leprae*.³³ These bacteria can be found ubiquitously in natural environments, in particular in soil, water, and dust; in contrast to MTB, they are usually not transmitted from person to person by direct contact.^{11,34} More than 190 species have been identified; among the clinically relevant ones are the *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*,

Mycobacterium abscessus, and *Mycobacterium fortuitum*.³⁵ They are classified as slow growers (e.g., *M. avium* and *M. kansasii* that take more than seven days to form colonies) or rapid growers (e.g., *M. abscessus* and *M. fortuitum* that take less than a week to form colonies).^{33,36} NTM mainly infects immunosuppressed persons, such as those with HIV, cystic fibrosis, or chronic lung disease (e.g., chronic obstructive pulmonary disease), although incidence in healthy individuals is rising.^{12,37} They are resistant to adverse conditions, such as low oxygen levels, heat, and disinfectants, which contribute to their survival in the hospital environment.^{11,38} They may also form biofilms on medical equipment and lung tissue, notably *M. abscessus*, which complicates therapy.^{39,40} Therapy is difficult because of their intrinsic resistance to several antibiotics due to their lipid-rich cell walls and strong efflux pumps.^{41,42}

Nontuberculous mycobacterial infections may manifest clinically as lung disease, soft-tissue infections, or disseminated disease, particularly in immunocompromised hosts.^{12,35} They are emergent public health problems due to their rising frequency, diagnostic limitations, and non-mandatory reporting in many places.^{9,43} The comparative features of MDRMTB and NTM are summarized in [Table 1](#).

Table 1. Comparison of MDR-MTB and NTM characteristics

Characteristics	MTB	NTM
Pathogen	Drug-resistant <i>Mycobacterium tuberculosis</i> (MTB) strains	Environmental mycobacteria (e.g., <i>Mycobacterium avium</i> , <i>Mycobacterium abscessus</i>)
Classification	Slow-growing; transmitted person-to-person	Includes slow growers (<i>M. avium</i> , <i>Mycobacterium kansasii</i>) and rapid growers (<i>M. abscessus</i> , <i>Mycobacterium fortuitum</i>); transmitted through the environment
Virulence factor	Lipid-rich cell wall; ESAT-6 and CFP-10 secreted via ESX-1 system; PPE2 suppresses ROS/NO; Zmp1 inhibits inflammasome	Biofilm production via GPL and PKS1; efflux pump systems; PtpA inhibits phagosome-lysosome fusion; LAM modulates cytokines
Clinical manifestations	Pulmonary TB with potential for latency and reactivation	Chronic lung infections (<i>M. abscessus</i> biofilms), disseminated disease in immunocompromised hosts (<i>M. avium</i> complex)
Immune evasion mechanisms	Blocks phagosome-lysosome fusion via SapM and ESAT-6; resists ROS/RNS through PPE2; evades AIM2 inflammasome via Zmp1; interferes with ubiquitination/autophagy	Biofilm-mediated shielding via GPL and PKS1; autophagy inhibition through PtpA and LAM; cytokine modulation (↓ TNF-α/IL-12, ↑ IL-10/TGF-β); granuloma persistence

Notes: SapM⁴⁴, ESAT-6³⁰, PPE2⁴⁵, and Zmp1⁴⁶ are key MDR-MTB mediators. For NTM, GPL⁴⁷, PKS1⁴⁸, PtpA⁴⁹, and LAM^{50,51} are representative examples. Abbreviations: AIM2: Absent in melanoma 2; CFP-10: Culture filtrate protein 10 kDa; ESAT-6: Early secretory antigenic target 6 kDa; ESX-1: ESAT-6 secretion system 1; GPL: Glycopeptidolipid; IL: Interleukin; LAM: Lipoarabinomannan; MDR: Multidrug resistance; NO: Nitric oxide; NTM: Nontuberculous mycobacteria; PKS1: Polyketide synthase 1; PPE2: Pro-Pro-Glu protein 2; PtpA: Protein tyrosine phosphatase A; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; SapM: Secreted acid phosphatase M; TB: Tuberculosis; TGF-β: Transforming growth factor beta; TNF-α: Tumor necrosis factor alpha; Zmp1: Zinc metalloprotease 1.

3. Immune evasion mechanisms

3.1. Immune evasion mechanisms in *Mycobacterium tuberculosis*

Multidrug-resistant MTB employs a variety of complex immune evasion strategies to survive, persist, and cause chronic infection in the host. These methods include preventing phagosome-lysosome fusion⁴⁴, resistance to reactive oxygen and nitrogen species⁵², regulation of host ubiquitination pathways⁵³, and interference with absent in melanoma 2 (AIM2)-mediated cytosolic DNA sensing.⁵⁴ Together, these methods allow pathogen escape from innate immune clearance and establishment of long-term intracellular infection.

3.1.1. Inhibition of phagosome-lysosome fusion

Following phagocytosis by macrophages, MTB is contained in early phagosomes that undergo a closely controlled maturation process. During normal endosomal maturation, the small GTPase RAB5 is recruited to early phagosomal membranes, where it mediates recruitment of early endosomal antigen 1 (EEA1). Phosphatidylinositol 3-phosphate (PI3P) is necessary for early endosomal identity and maturation and functions as a major docking site for EEA1.

As maturation progresses, RAB5 is gradually replaced by RAB7, reflecting the change from early to late phagosomes. This modification of RAB is an essential stage in endosomal trafficking and is necessary for subsequent fusion with lysosomes. Late endosomes and lysosomes are loaded with membrane-associated proteins, such as lysosome-associated membrane protein 1 (LAMP1), LAMP2, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes, and vacuolar ATPase (vATPase) that acidify the compartment and facilitate enzymatic destruction of imported pathogens (Figure 1).^{55,56}

However, MTB aggressively intervenes in this maturation mechanism. One important virulence-associated mechanism is the SapM, which dephosphorylates PI3P on the phagosomal membrane. This loss of PI3P hinders the proper recruitment of RAB7 and EEA1-associated maturation processes, hence stopping phagosome growth at an early step. RAB7 acquisition is needed for the transition from early to late phagosome and subsequent fusion with the lysosome; hence, its inhibition efficiently limits phagolysosome formation (Figure 2).^{44,57}

Experiments provide evidence of this process. Vergne *et al.*⁵⁷ showed that SapM inhibits PI3P-dependent phagosome-late endosome fusion in an in vitro organelle

fusion experiment. In this system, we quantified membrane fusion by measuring horseradish peroxidase (HRP) transfer from streptavidin bead-containing phagosomes to biotin-HRP-loaded late endosomes. Addition of pure SapM strongly inhibits ATP-dependent phagosome-late endosome fusion, whereas the control proteins glutathione S-transferase (GST), mycobacterium protein tyrosine phosphatase A (MptpA), and MptpB show no inhibition. Importantly, suppression of phosphoinositide 3-kinase (PI3K) activity by wortmannin verified the PI3P dependence of this fusion process. Together, our results show that SapM-mediated PI3P depletion is a major immune evasion mechanism to block phagosome formation and lysosomal destruction of MTB.⁴⁴

The majority of this mechanistic understanding has come from laboratory reference strains such as H37Rv, whose SapM activity has been experimentally confirmed in vitro.^{44,57} Evidence from clinical MDR-MTB isolates is relatively restricted; however, SapM expression has been observed across a range of patient-derived strains, indicating the conservation of this pathogenicity mechanism.²⁷ Direct evidence of PI3P depletion in clinical isolates is lacking, suggesting a significant extrapolation of existing knowledge from reference models. Further research is needed to demonstrate SapM activity in MDR-MTB clinical isolates and evaluate its functional effect in patient infections.

3.1.2. Resistance to reactive oxygen and nitrogen species

Macrophages are important immune cells that ingest MTB via phagocytosis, forming a phagosome that generally evolves into a phagolysosome to destroy and lyse the bacterium. Macrophages kill microorganisms by oxidative and nitrosative bursts. This involves the generation of reactive oxygen species (ROS) by NADPH oxidase 2 (NOX2) and nitric oxide (NO) by inducible nitric oxide synthase (iNOS; encoded by the *Nos2* gene). ROS and NO are powerful antibacterial agents that damage bacterial cellular components, such as proteins, lipids, and DNA (Figure 3).

Mycobacterium tuberculosis has evolved mechanisms to thrive within macrophages, thereby counteracting the harsh environment. PPE family proteins of mycobacteria have been proposed to operate as virulence factors or immune modulators by blocking host signal transduction pathways. As shown by Bhat *et al.*⁴⁵, PPE2 is a vital component of MTB survival. PPE2 is a secretory protein that is translocated from the bacteria in the phagosome to the host macrophage nucleus. The nuclear localization of PPE2 is an interesting result, suggesting that PPE2 may

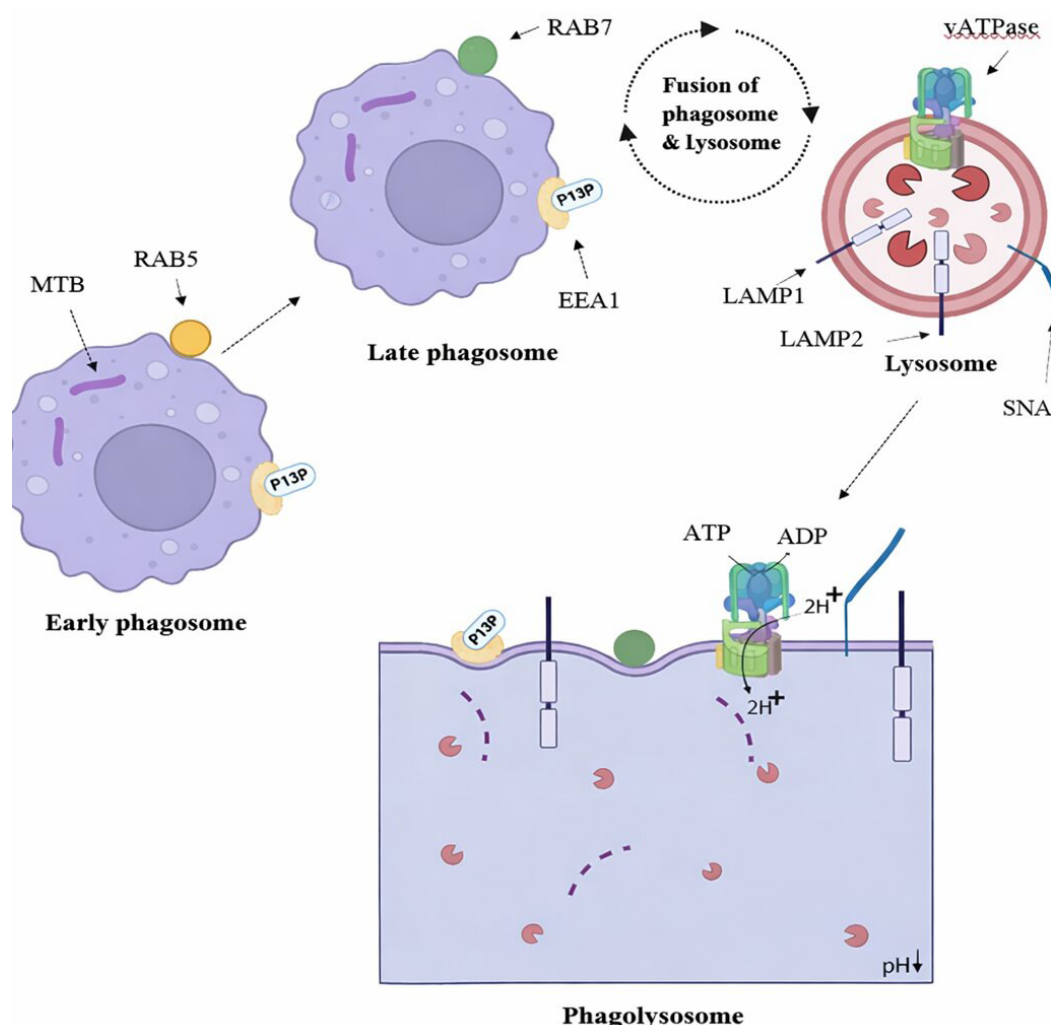


Figure 1. Phagolysosomal fusion

Abbreviations: EEA1: Early endosomal antigen 1; LAMP1: Lysosome-associated membrane protein 1; MTB: *Mycobacterium tuberculosis*; RAB5: Ras-related protein Rab-5; SNARE: Soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

directly interfere with host gene expression. In particular, PPE2 suppresses the *Nos2* gene, preventing NO production and consequently reducing nitrosative stress on MTB.^{45,58} The inhibition of NO is a well-established mechanism, supported by experimental data showing PPE2-mediated downregulation of *Nos2* and decreased iNOS activity (Figure 4).

The PPE2 protein also prevents ROS production. However, how this happens remains unclear. PPE2 has been shown to modulate oxidative burst reactions, but current interpretations are speculative. The possibility of disruption of NOX2 complex assembly has been hypothesized but not experimentally proven. Thus, ROS inhibition is an inferred mechanism that has to be validated.⁴⁵

3.1.3. Evasion of the ubiquitination pathway

The MTB DNA and the host proteins, p62 and nuclear domain 10 protein 52 (NDP52), are detected in the phagosome. p62 (encoded by *SQSTM1*) and NDP52 (encoded by *CALCO2*) are autophagy adaptor proteins that bind ubiquitinated cargo and target it for destruction by autophagy. MTB may disrupt the phagosomal membrane via its ESAT-6 secretion system 1 (ESX-1), which releases bacterial components, such as DNA, into the cytosol. p62 and NDP52 have been shown to bind ubiquitinated MTB, targeting them for autophagic destruction (xenophagy).⁵⁹

The type VII secretion system, ESX-1, is a major virulence factor in MTB that damages the phagosomal membrane,

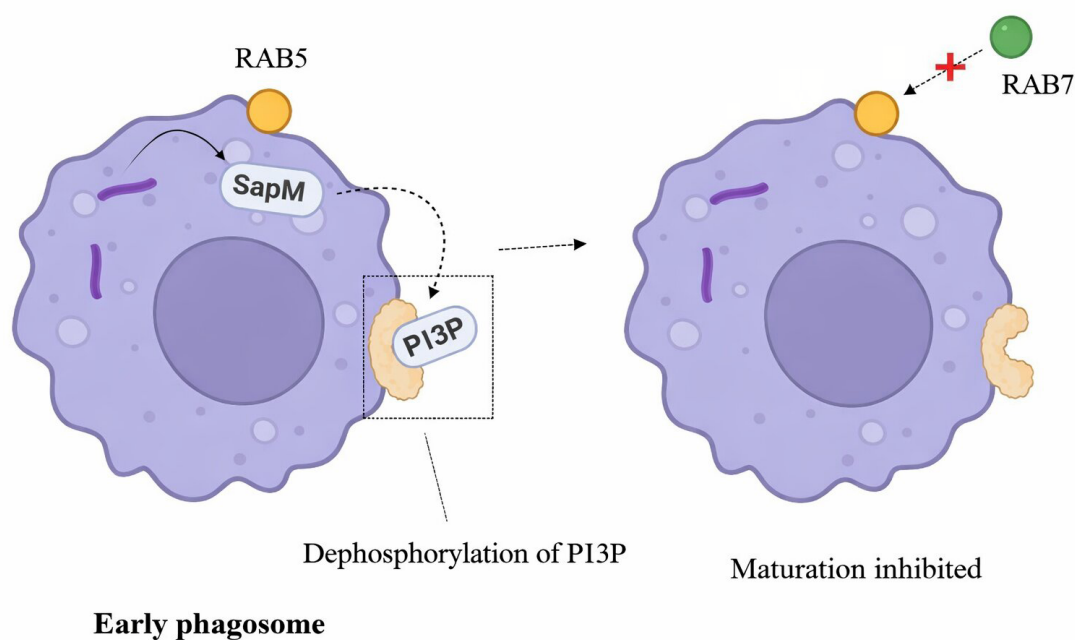


Figure 2. Phagolysosomal fusion evasion

Abbreviations: PI3P: Phosphatidylinositol 3-phosphate; RAB5: Ras-related protein Rab-5; SapM: Secreted acid phosphatase M.

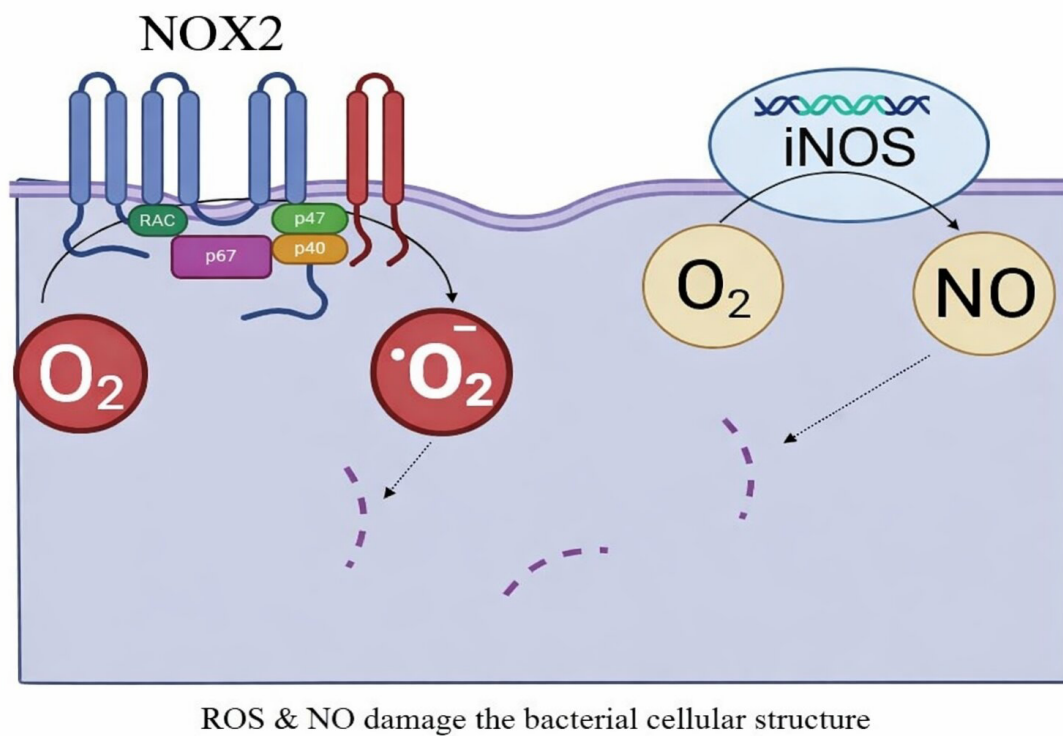


Figure 3. ROS activation

Abbreviations: iNOS: Inducible nitric oxide synthase; NO: Nitric oxide; NOX2: NADPH oxidase 2; ROS: Reactive oxygen species.

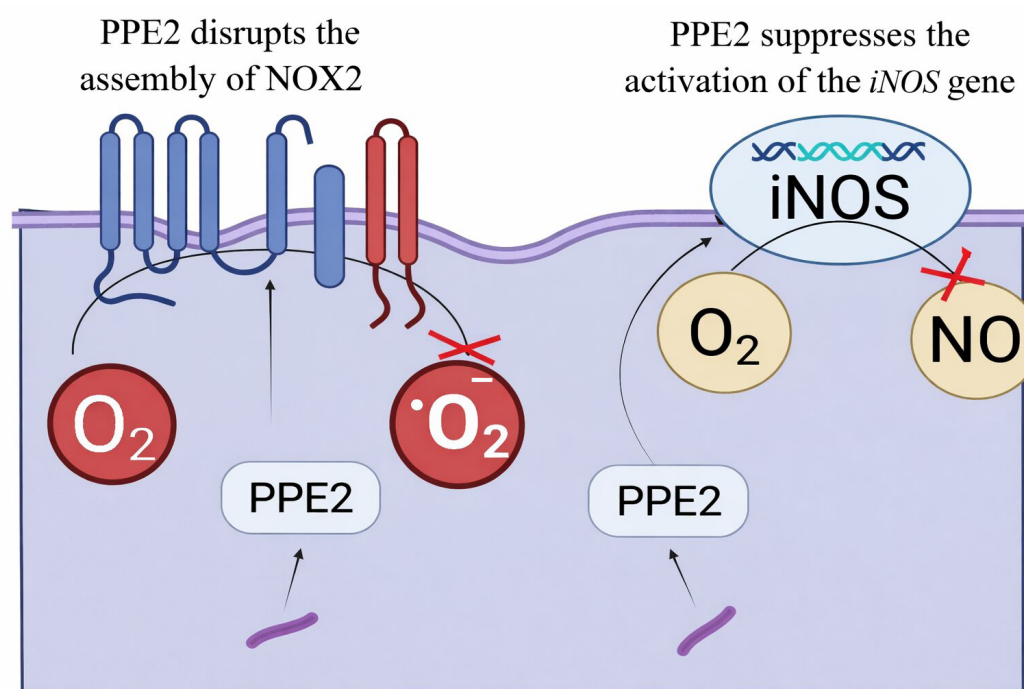


Figure 4. ROS deactivation

Abbreviations: iNOS: Inducible nitric oxide synthase; NO: Nitric oxide; NOX2: NADPH oxidase 2; PPE2: Pro-Pro-Glu protein 2; ROS: Reactive oxygen species.

leading to the release of bacterial DNA into the cytosol.⁶⁰ cGAS detects cytosolic DNA and creates cyclic GMP-AMP (cGAMP) that activates the STING pathway and induces type I interferon (IFN) responses.¹⁷ This route is well known in infection with MTB. Galectin-8 has been shown to bind to damaged phagosomal membranes and to attract adaptor proteins, such as NDP52, to initiate autophagy. It is a lectin that identifies exposed β -galactoside residues on damaged membranes for autophagic clearance. Galectin-8 primarily promotes autophagy via adaptor recruitment, while STING primarily mediates IFN signaling. However, evidence suggests that STING may interact with autophagy pathways, and galectin-8 can indirectly increase STING-mediated responses by facilitating the clearance of injured phagosomes (Figure 5).⁶¹

Ubiquitination is a crucial signaling pathway that marks pathogens or damaged organelles for autophagic breakdown (xenophagy). p62 and NDP52 recognize ubiquitinated targets and recruit autophagosomal membranes to engulf MTB.⁵⁹ Ubiquitin conjugation occurs via the E1-E2-E3 cascade, and E3 ligases, such as Parkin and tripartite motif-containing protein (TRIM) proteins, have been shown to ubiquitinate MTB or related phagosomal membranes.⁶² Deubiquitinating enzymes (DUBs) cleave ubiquitin tags

from MTB or phagosomal membranes and antagonize host ubiquitination, thereby blocking the recruitment of autophagy adaptors, such as p62 and NDP52, and impeding autophagic breakdown. Although MTB does not encode DUBs, it may subvert host DUBs or release effectors that imitate DUB activity to circumvent xenophagy.^{53,59,60} MTB effectively evades the ubiquitination mechanism by facilitating deubiquitination. Specific host DUBs participating in this mechanism have been discovered in recent experimental investigations. Ubiquitin-specific peptidase 10 (USP10) and cylindromatosis (CYLD) have been shown to remove ubiquitin tags from phagosomal substrates, thereby promoting bacterial viability and blocking autophagy adaptor binding (Figure 6).^{63,64} The inclusion of these cases suggests that MTB not only depends on generic deubiquitination but may also exploit specific host DUBs to persist in macrophages. This allows MTB to keep influencing the host cell, secreting more virulence factors and perhaps escaping into the cytosol or moving to other cells.⁶⁵ This is further supported by the elimination of ubiquitin tags and other evasion tactics such as protein tyrosine phosphatase A (PtpA) and PE_PGRS47 (which interfere with autophagy and phagosome maturation), which contribute to the long-term survival of MTB.^{49,66} Defective autophagic clearance is a major cause of chronic

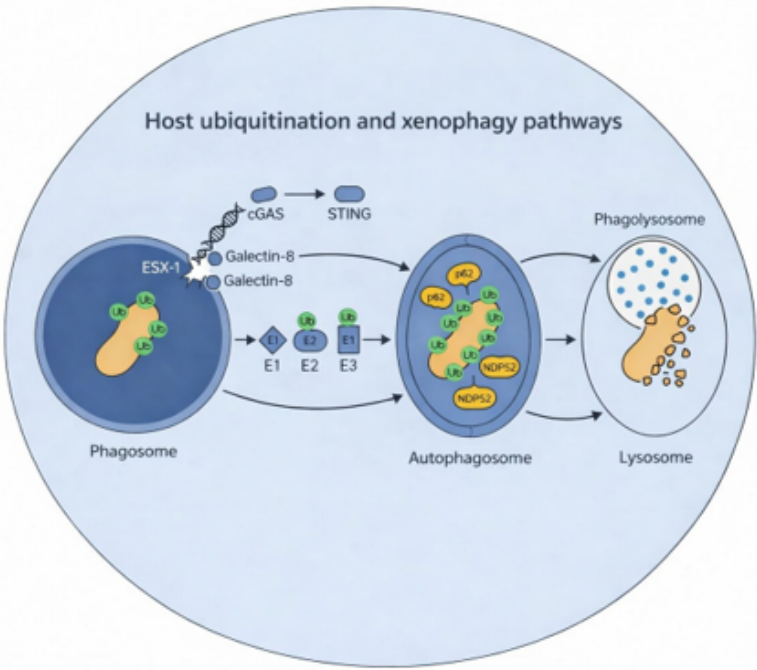


Figure 5. Ubiquitination pathway
Abbreviations: cGAS: Cyclic GMP–AMP synthase; ESX1: ESAT-6 secretion system 1; NDP52: Nuclear domain 10 protein 52; STING: Stimulator of interferon genes.

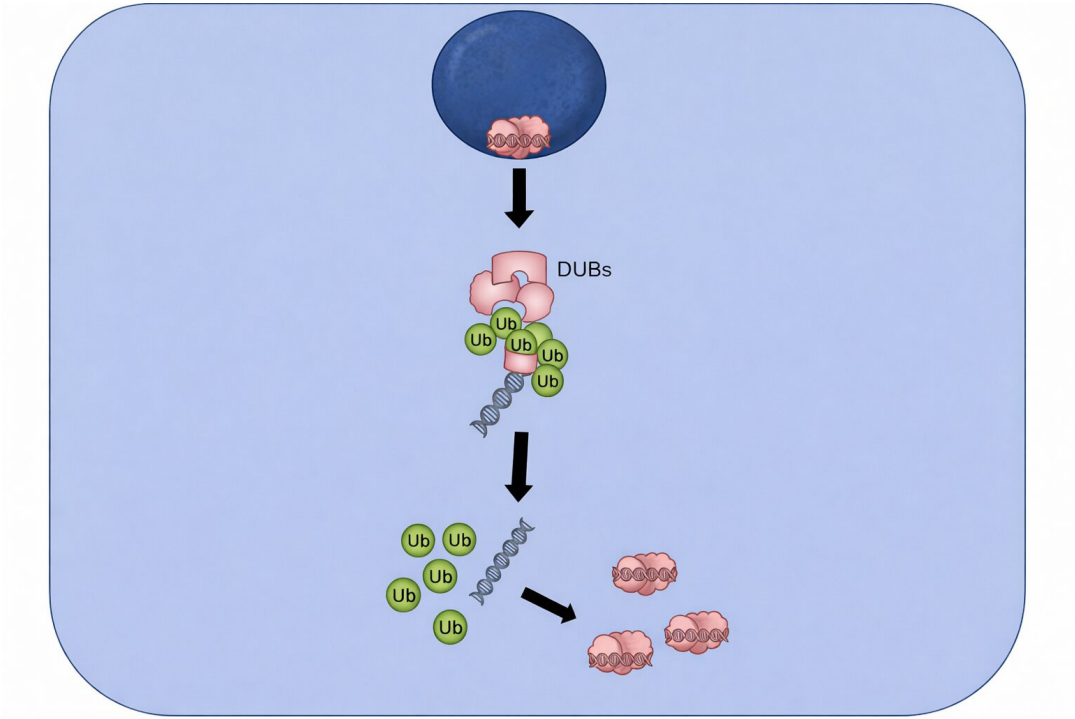


Figure 6. Ubiquitination evasion
Abbreviation: DUB: Deubiquitinating enzyme.

infection. Macrophages may be a latent reservoir for MTB, where bacteria can lie dormant or grow slowly, eventually leading to spread and disease development, including granuloma formation and TB reactivation.⁶⁷

3.1.4. AIM2-driven cytosolic DNA sensing

Mycobacterium tuberculosis is engulfed by macrophages during phagocytosis. MTB use ESX-1, a critical virulence factor, to damage phagosomal membrane integrity and release bacterial DNA into the cytosol. Cytoplasmic DNA is recognized by the double-stranded DNA sensor AIM2, which induces the formation of the AIM2 inflammasome. AIM2 recruits apoptosis-associated speck-like protein containing a CARD (ASC), which binds pro-caspase-1 via its CARD domain to create the full inflammasome complex. Within this assembly, pro-caspase-1 is cleaved to its active form, caspase-1, which subsequently cleaves the pro-inflammatory cytokines pro-interleukin (IL)-1 β and pro-IL-18 to their active forms, IL-1 β and IL-18, respectively. Moreover, caspase-1 cleaves gasdermin D, releasing its N-terminal fragment that generates membrane holes in macrophages and triggers pyroptosis, an inflammasome-dependent programmed cell death. This cell death and cytokine release activate the immune system

and improve host resistance against MTB (Figure 7).

However, MTB has evolved strategies to bypass this defense. It produces Zmp1, which suppresses caspase-1 activation by interfering with proper inflammasome formation or function.^{68,69} Pro-caspase-1 is therefore not activated, and cleavage of pro-IL-1 β and pro-IL-18 into their mature, inflammatory forms is inhibited. This inhibition reduces IL-1 β and IL-18 production and pyroptosis, enabling MTB to survive and replicate inside host cells (Figure 8). This immune evasion approach allows MTB to develop long-term persistence in the host with depressed inflammatory responses and cell death that might typically contribute to bacterial elimination.

Recent findings suggest that AIM2 signaling may vary across MTB lineages. In murine models, AIM2 loss is associated with decreased secretion of IL-1 β and IL-18 and greater susceptibility to MTB, emphasizing its crucial role in host defense.⁶⁹ In contrast, in human macrophages, AIM2 induction is lineage-dependent and generally less crucial than NLRP3 to triggering IL-1 β release, with ESX-1 not necessarily necessary for AIM2 activation.⁵⁴ Comparable data for NTM species, which include *Mycobacterium smegmatis* and *Mycobacterium*

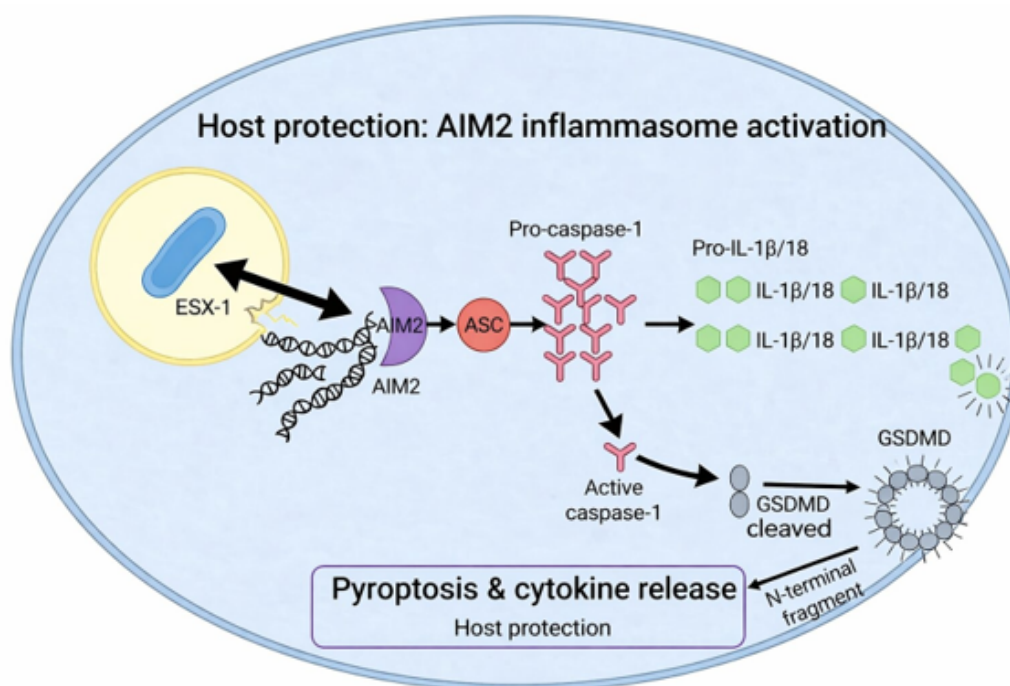


Figure 7. AIM2 pathway

Abbreviations: AIM2: Absent in melanoma 2; ASC: Apoptosis-associated speck-like protein containing a CARD; ESX-1: ESAT-6 secretion system 1; GSDMD: Gasdermin D; IL: Interleukin.

MTB evasion: Zmp1 inhibition

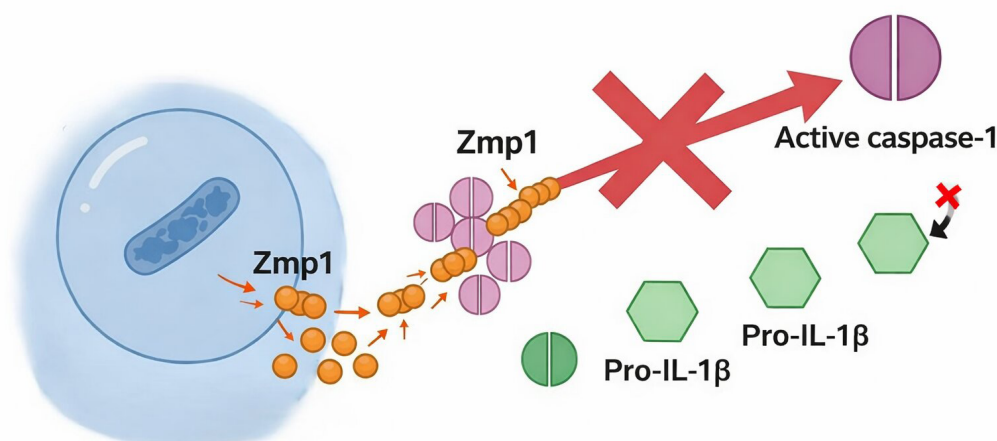


Figure 8. AIM2 evasion pathway

Abbreviations: AIM2: Absent in melanoma 2; IL: Interleukin; MTB: *Mycobacterium tuberculosis*; Zmp1: Zinc metalloprotease 1.

bovis BCG, show that AIM2 may be activated, but its role in cytokine release is less prominent, with NLRP3 being more dominant in human macrophages.^{54,69}

In addition to AIM2, several NTM species also partially activate the cGAS-STING pathway. For example, *M. smegmatis* and *M. bovis* BCG may stimulate cGAS-STING signaling in human macrophages, with modest type I IFN responses, and *M. abscessus* has already been shown to partially degrade phagosomal membranes and release DNA into the cytosol, resulting in weaker STING-dependent activation than MTB.^{54,70} These results reveal that whereas MDR-MTB actively inhibits cGAS-STING via effectors such as Zmp1, NTM exhibit heterogeneous, often diminished interactions with cytosolic DNA-sensing pathways, rather than a universal evasion.

3.2. Immune evasion mechanisms in nontuberculous mycobacteria

3.2.1. Evasion of autophagy and phagocytosis

(a) Host immune response

Nontuberculous mycobacteria are internalized by phagocytes. Interactions between NTM and phagocytes, such as macrophages, begin when macrophages recognize NTM via pattern recognition receptors that bind pathogen-associated molecular patterns, such as lipoarabinomannan (LAM), leading to phagocytosis and the formation of a phagosome.⁷¹ RAB5 and then RAB7 vesicular trafficking

GTPases are recruited sequentially to develop the phagosome. This process finally results in fusion of the phagosome with lysosomes and development of a phagolysosome, where the acidic pH, hydrolytic enzymes, and ROS produce a microenvironment deadly to NTM.⁷²

The degraded microbial components are then processed into peptides and loaded onto major histocompatibility complex (MHC) class II molecules for presentation to CD4+ T cells or cross-presented via MHC class I molecules to CD8+ T cells, activating adaptive immune responses using antigen-presenting cells (APCs).⁷³

(b) Nontuberculous mycobacteria evasion mechanism

Nontuberculous mycobacteria bypass the host immune response by secreting virulence factors, including SapM, PtpA, and LAM, into the phagosome, thereby jointly blocking phagolysosomal fusion.^{44,49,56} SapM dephosphorylates the PI3P on the phagosomal membrane, which is necessary for RAB7 recruitment. PtpA dephosphorylates host proteins, including vacuolar protein sorting-associated protein 33B (VPS33B), to block the homotypic fusion and protein sorting (HOPS) complex required for lysosomal fusion and LAM to inhibit calcium signaling to facilitate PI3P accumulation.^{44,49,55,56} These substances limit phagosome maturation and lysosome fusion by preventing RAB7 recruitment, so NTM may survive in a non-degradative phagosome and possibly reproduce, providing a site of persistent infection.^{67,74}

However, these processes are not equally preserved across all NTM species. There is evidence that SapM and PtpA activities are widely established in clinically significant pathogens, including MAC and *M. abscessus*, while LAM-mediated inhibition is more evident in slow-growing NTM, such as *M. kansasii* and MAC.^{70,75} In contrast, rapidly growing NTM, such as *M. fortuitum* and *Mycobacterium chelonae*, depend more on biofilm formation and efflux pumps than on inhibiting phagolysosomal fusion.⁴⁷ Thus, SapM, PtpA, and LAM represent conserved techniques in numerous pathogenic NTM, although their proportional contribution differs by species, suggesting ecological variety and clinical importance (Figure 9).

3.2.2. Granuloma formation

Nontuberculous mycobacteria infection induces granuloma formation, with macrophages, CD4+ T cells, multinucleated giant cells (MGCs), and regulatory T cells, which help regulate infection and thereby favor persistence.^{76,77} After identification of NTM, macrophages initiate phagocytosis, and the bacteria are ingested. This results in activation and production of cytokines, such as IL-2, tumor necrosis factor alpha (TNF- α), and IFN- γ .⁷⁸ IL-2 is involved in T-cell proliferation; TNF- α and IFN- γ stimulate macrophages and attract other immune cells to the site of infection. The macrophage presents NTM

antigens to an APC, which activates CD8+ T cells and initiates an immune response.⁷⁹ This activation amplifies the immune response, recruiting additional immune cells, such as monocytes and neutrophils, to contain the infection.⁷⁶

During the immune response, a granuloma contains NTM to prevent its spread and consists of an organized collection of immune cells.⁷⁷ Differentiating monocytes become macrophages, and these, together with CD4+ T cells, constitute the central core of the granuloma, attempting to restrain infection through phagocytosis and cytokine secretion.⁷⁶ In early inflammation, neutrophils also play a role. At the same time, fibroblasts produce an extracellular matrix that forms a fibrotic capsule around the granuloma, and multinucleated giant cells (MGCs) develop through macrophage fusion to enhance bacterial containment.⁷⁷ Regulatory T cells modulate immune responses to prevent excessive inflammation, ensuring that the granuloma optimizes containment of NTM while minimizing tissue damage (Figure 10).⁷⁹

3.2.3. Biofilm formation and persistence

(a) Early attachment and extracellular matrix production

Biofilm formation by NTM is mediated by glycopeptidolipid (GPL) and polyketide synthase 1 (PKS1), and polyphosphate

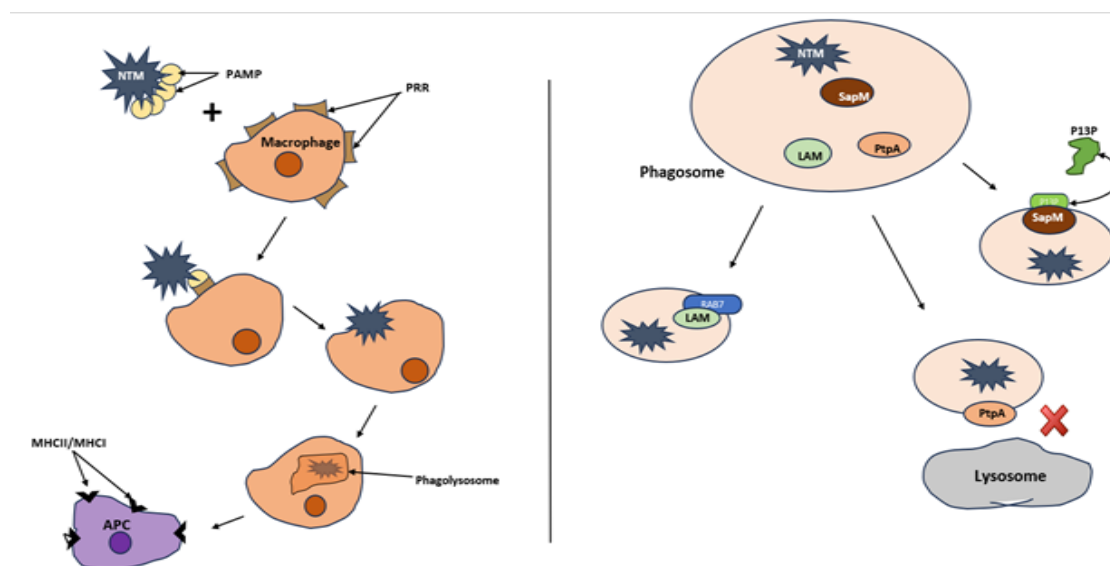


Figure 9. NTM evasion mechanism

Abbreviations: APC: Antigen-presenting cell; LAM: Lipoarabinomannan; NTM: Nontuberculous mycobacteria; PAMP: Pathogen-associated molecular pattern; PI3P: Phosphatidylinositol 3-phosphate; PRR: Pattern recognition receptor; PtpA: Protein tyrosine phosphatase A; SapM: Secreted acid phosphatase M.

plays a role in NTM survival under stress.^{47,48,80} NTM causes biofilm development by attaching the standard molecule to the bronchial epithelium of lung tissue via GPL, thereby enhancing adherence to host surfaces and supporting colonization.⁴⁷ During attachment, NTM starts secreting an extracellular matrix of lipids, proteins, and carbohydrates that constitute the structural foundation of the biofilm. This matrix surrounds the bacteria, shielding them from human immunological responses and environmental stress.⁸⁰ This matrix formation allows the NTM to create a stable community within the lung tissue, which, in turn, promotes the continued growth of biofilms.

(b) Maturation and maintenance

Polyketide synthase 1 is overexpressed by NTM during maturation and contributes to the production of lipid components that stabilize the extracellular matrix, thereby preserving the structural integrity of the biofilm.⁴⁸ The buildup of polyphosphate within the biofilm further aids maintenance by boosting bacterial survival under stress circumstances, such as food limitation and antibiotic exposure, and promoting persistence in the lung.⁸⁰ This developed biofilm architecture allows NTM to escape human defense and antimicrobial treatment, leading to the persistent infections typical of NTM lung disease (Figure 11).

Recent findings indicate that biofilm control differs among therapeutically important NTM species. In *M. abscessus*, biofilm formation is highly reliant on the synthesis of GPL and quorum-sensing-like pathways, and two-component regulatory systems respond to the host lung environments.^{81,82} In contrast, biofilm control in *M. avium* is associated with lipid metabolism, iron uptake, and stress-response sigma factors that regulate biofilm thickness and antibiotic tolerance.⁸³ Other NTM species, such as *M. kansasii*, depend more on oxidative stress regulators and alternative transcriptional programs rather than GPL-driven pathways.⁷⁰ These discrepancies suggest that whereas biofilm production is a preserved persistence mechanism, the regulatory channels are species-specific and represent natural habitats and pathogenic tactics.

These variable differences in regulation not only determine biofilm construction and antibiotic resistance but also identify species-specific treatment targets, emphasizing the necessity of precision methods in NTM management.³⁹

3.2.4. Modulation of cytokine responses

Nontuberculous mycobacteria downregulate TNF- α and IL-12 via LAM and TLR2 inhibition and upregulate IL-10 and transforming growth factor beta (TGF- β) to create

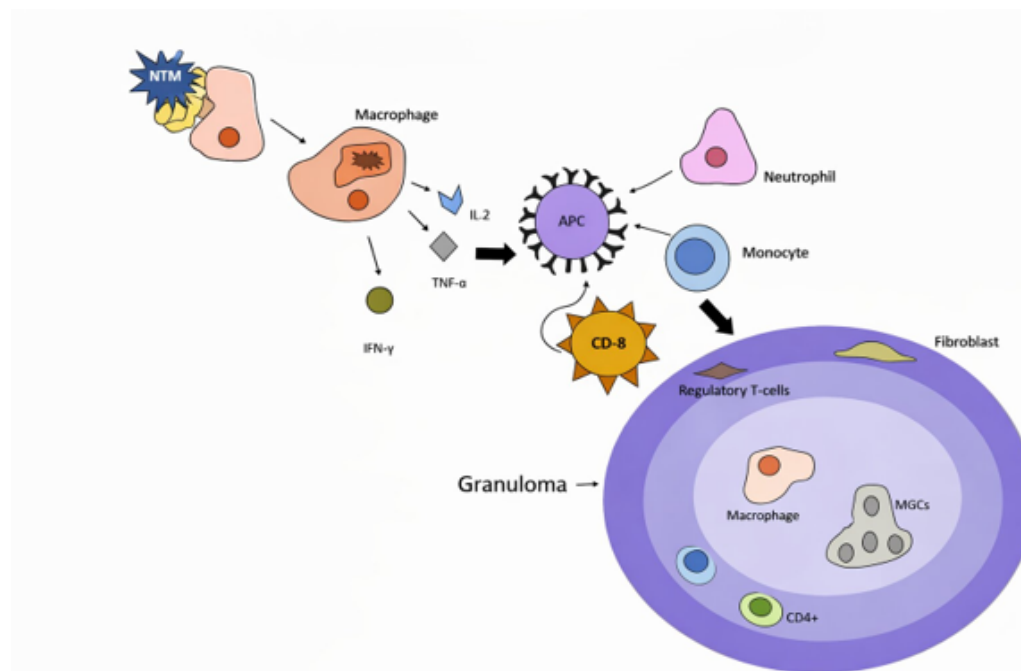


Figure 10. Granuloma Formation

Abbreviations: APC: Antigen-presenting cell; IFN- γ : Interferon gamma; IL: Interleukin; MGC: Multinucleated giant cell; NTM: Nontuberculous mycobacteria; TNF- α : Tumor necrosis factor alpha.

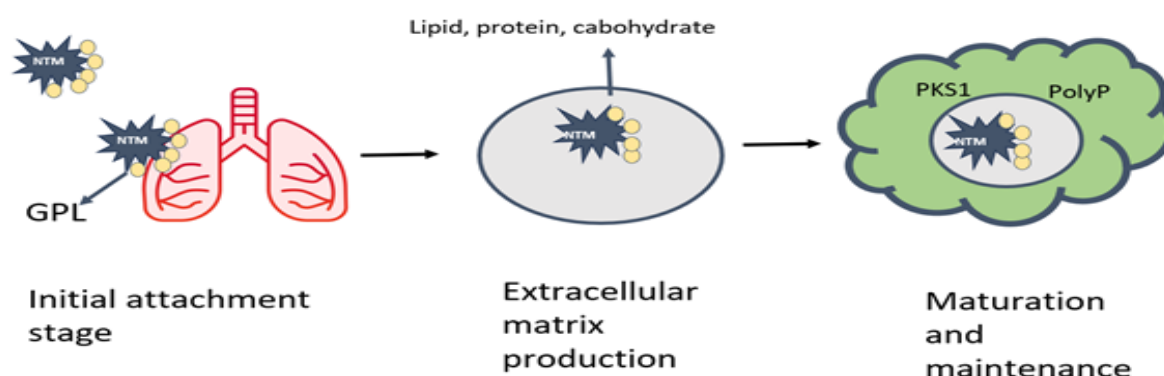


Figure 11. Biofilm production of NTM on lung tissues

Abbreviations: GPL: Glycopeptidolipid; NTM: Nontuberculous mycobacteria; PKS1: Polyketide synthase 1; PolyP: Polyphosphate.

an immunosuppressive environment.^{50,51} The modulation of cytokine responses by NTM is an elaborate system by which these germs evade or reduce effective immune responses to facilitate bacterial survival. The pathways by which NTM control cytokine production are essential for the activation and coordination of immune cells, such as macrophages and T cells.

(a) Suppression of pro-inflammatory cytokines

After infection, NTM is taken up by macrophages, which would ordinarily initiate a pro-inflammatory response by secreting cytokines such as TNF- α , IL-1 β , and IL-12 to activate immune responses and attract additional immune cells.⁷⁸ NTM subverts this response by generating virulence factors, such as LAM, that disrupt TLR signaling, most notably TLR2, thereby decreasing TNF- α and IL-12 release, macrophage activation, and the inflammatory response.⁵⁰ NTM also inhibits IFN- γ release from T cells as another defect that blocks macrophage activation and the Th1-mediated immunity critical for controlling mycobacterial infection.⁸⁴

(b) Manipulation of anti-inflammatory responses

Nontuberculous mycobacteria induce anti-inflammatory cytokines, including IL-10 and TGF- β , to escape host immunity, thereby inhibiting pro-inflammatory responses and allowing bacterial persistence.⁵¹ IL-10, induced by NTM through mechanisms involving cell wall components, such as LAM, suppresses the synthesis of pro-inflammatory cytokines and downregulates MHC class II expression on macrophages, thereby impairing antigen presentation and T-cell activation.⁵⁰ NTM also induce TGF- β , which

suppresses macrophage activation and boosts regulatory T-cell responses, creating an immunosuppressive milieu for NTM to survive inside the host and resulting in chronic infection.⁸⁴

However, observations on cytokine modulation are inconsistent and vary with NTM species and host cell type. For example, *M. avium* has been reported to stimulate substantial release of TNF- α and IL-12 in dendritic cells and macrophages^{83,85}, whereas *M. abscessus* typically decreases TNF- α production in macrophages, especially under IFN- γ priming conditions.⁷⁵ *M. kansasii* has a differential IL-12 induction profile in alveolar macrophages compared to peripheral blood monocytes.⁵¹ Human monocyte-derived macrophages also tend to demonstrate a greater stimulation of IL-10 than macrophages from mice. Dendritic cells exposed to NTM may release more IL-12 and TNF- α , suggesting cell-specific variations. These data demonstrate that cytokine regulation is not generally conserved but rather depends on the infecting NTM species and the host cell type.

4. Comparative analysis

4.1. Similarities: Common strategies used by *Mycobacterium tuberculosis* and nontuberculous mycobacteria

Multidrug-resistant MTB and NTM share immune evasion mechanisms that allow them to persist in the host, thereby making therapy difficult and contributing to pathogenicity. Both MDR-MTB and NTM block phagosome-lysosome fusion, modify cytokine responses, develop granulomas,

and are resistant to ROS/reactive nitrogen species (RNS) owing to their lipid-rich cell walls.^{28,30,52,57,79,86}

Their common immune evasion mechanisms include:

- Blocking phagosome–lysosome fusion: MDR-MTB and NTM alter phagosome development in macrophages to prevent degradation. MDR-MTB suppresses RAB7 recruitment via ESAT-6⁸⁶, and NTM alters lysosomal trafficking through factors such as SapM and PtpA.⁵⁷
- Modulation of cytokines: MDR-MTB and NTM modify the cytokine responses by downregulating pro-inflammatory mediators, including TNF- α and IL-12, via LAM, and upregulating IL-10 to create an immunosuppressive environment.^{30,87}
- Granuloma formation: Both species induce the development of granulomas by macrophages, T cells, and fibroblasts, generating a structured niche that restricts immune access and provides protection against antibiotics.^{79,88}

Their structural and resistance mechanisms include:

- Lipid-rich cell wall: Lipid cell wall with a high concentration of mycolic acid in MDR-MTB and NTM is a formidable barrier to immune clearance and antimicrobial agents.^{19,28}
- Resistance to ROS and RNS: MDR-MTB and NTM produce enzymes, such as superoxide dismutase and catalase, that scavenge ROS and RNS produced by host defenses.^{52,89}

These common processes, taken together, underscore the difficulty of treating mycobacterial infections and point to the need for targeted medicines to counter their sophisticated immune evasion strategies.

4.2. Differences: Unique mechanisms employed by *Mycobacterium tuberculosis* and nontuberculous mycobacteria

Multidrug-resistant MTB uniquely evades cGAS–STING signaling, exploits the ESX-1 system (ESAT-6/CFP-10), and induces latency, whereas NTM rely more on biofilm formation, efflux pumps, and environmental resilience for survival.^{19,30,90–93}

Specifically, MDR-MTB's unique mechanisms include:

- cGAS–STING pathway evasion: MDR-MTB evades detection by the innate immune system by secreting proteins, such as lipoprotein G and protein kinase G, that alter phagosomal trafficking and disrupt normal cellular processes with STING activation to limit IFN- β generation.⁹⁰

- ESAT-6 and CFP-10 secretion: MDR-MTB secretes ESAT-6 and CFP-10 via the ESX-1 secretion system. These factors hinder immunological signaling by stimulating macrophage death and blocking antigen presentation.³⁰
- Latency and reactivation: MDR-MTB is known to generate a latent infection via metabolic incubation and to evade immune detection and reactivate in immunocompromised states, a property not often shared by NTM.⁹¹

Meanwhile, NTM's unique mechanisms include:

- Biofilm formation: NTM, especially *M. abscessus*, form protective biofilms via GPL and PKS1 on lung tissue and medical equipment, resulting in drug resistance and immune evasion.¹⁹
- Efflux pump activity: NTM has very strong intrinsic resistance via active drug efflux pumps, notably the resistance–nodulation–division (RND) family, providing high-level resistance to macrolides and other antibiotics, more so than in MDR-MTB.⁹²
- Environmental resilience: NTM, unlike MDR-MTB, can survive in environmental reservoirs and are resilient to stresses such as low oxygen, heat, and disinfectants, allowing them to persist in healthcare and water systems.⁹³
- Interaction with DNA-sensing pathways: Recent research reveals that certain NTM species may interact with cytosolic DNA-sensing. For example, human macrophages enhance cGAS–STING signaling in response to *M. smegmatis* and *M. bovis* BCG, but to a lesser extent than MDR-MTB.⁵⁴ Slow-growing organisms, such as *M. kansasii*, also trigger STING-dependent signaling.⁷⁰

Thus, in contrast to MDR-MTB, which actively inhibits cGAS–STING via effectors such as Zmp1, NTM species do not uniformly evade but variably engage. These differential methods reflect each pathogen's biological adaptations and highlight the need for individualized diagnostic and treatment strategies targeting its specific pathogenic activity.

4.3. Implications for host–pathogen interactions: How these mechanisms influence disease progression and outcomes

The chronic characteristics, immune suppression, and diagnostic difficulty of MDR-MTB, along with the biofilm-driven, persistent nature and opportunistic behavior of NTM, highlight the need for tailored therapy.^{16,29,39,88,94}

4.3.1. Implications for multidrug-resistant *Mycobacterium tuberculosis*

The implications include:

- Chronic infection and latency: MDR-MTB remains in macrophages by suppressing RAB7 recruitment via ESAT-6, thereby preventing phagosome–lysosome fusion and prolonging infection.¹⁶ This potential explains latent infection and reactivation, notably in the immunocompromised.⁹¹
- Diagnostic and vaccine development hurdles: Antigenic variation and the protective lipid-rich cell wall mask promising diagnostic indicators and vaccine targets, impeding the development of effective tools.²⁹
- Immunosuppressive modulation: MDR-MTB secretes molecules, such as ESAT-6 and CFP-10, that inhibit pro-inflammatory signaling and promote IL-10 production via LAM, promoting persistence and spread.⁹⁴

4.3.2. Implications for nontuberculous mycobacteria

The implications include:

- Biofilm-associated chronicity: NTM, particularly *M. abscessus*, develop biofilms via the GPL and PKS1 pathways, particularly in cystic fibrosis patients, thereby conferring resistance to antibiotics and immunological assaults.³⁹
- Opportunistic infection in vulnerable hosts: NTM has an affinity for hosts with compromised immunity, such as those with HIV or cystic fibrosis, and is resistant to antimicrobial peptides and suppresses autophagy via SapM.⁹²
- Immune modulation: Like MDR-MTB, NTM suppresses TNF- α and increases IL-10 production via LAM and disruption of the TLR pathway, thereby establishing a favorable environment for chronic infection.⁸⁸

4.3.3. Shared implications

Therapeutic resistance shares evasion strategies, including inhibition of phagosome–lysosome fusion and cytokine suppression, as well as NTM-specific biofilm development that contributes to antimicrobial resistance and extensive, sometimes hazardous treatment regimens.⁸

The shared implications include:

- Vaccine development obstacles: Immune evasion through antigenic masking in MDR-MTB and biofilm protection in NTM pose major challenges to establishing durable vaccination responses.⁹⁵
- Host susceptibility and personalized medicine: Host

genetic variations, particularly in cytokine pathways, influence disease severity and outcomes, underscoring the value of precision medicine in managing these infections.⁹⁶

Understanding these complex interactions is essential for designing targeted therapies and preventive strategies to mitigate the global burden of MDR-MTB and NTM.

5. Clinical and therapeutic implications

Multidrug-resistant TB treatment is hindered by poor efficacy, toxicity, and delayed diagnosis, while NTM's innate resistance, inconsistent drug response, and biofilm formation complicate management.^{25,35,41,97–99}

5.1. Challenges in treating multidrug-resistant tuberculosis and nontuberculous mycobacteria infections

5.1.1. Multidrug-resistant tuberculosis

The therapeutic and diagnostic landscape in MDR-TB is beset with obstacles that complicate patient management and outcome prediction. Therapeutically, the use of second-line drugs presents several barriers, and diagnostically, detection of resistance is often delayed or unavailable in many healthcare facilities. The major obstacles include:

- Limited efficacy with second-line agents: Second-line agents, such as aminoglycosides (e.g., amikacin) and fluoroquinolones, are less effective than first-line agents, necessitating longer treatment durations of 18–24 months and an increased risk of therapeutic failure.⁹⁷
- Severe toxicity and side effects: These medications are associated with significant adverse effects, such as hepatotoxicity (e.g., ethionamide-induced liver damage), ototoxicity (e.g., aminoglycoside-induced hearing loss), nephrotoxicity (e.g., capreomycin-induced kidney damage), and neuropsychiatric effects (e.g., cycloserine-induced depression), all of which contribute to poor adherence and treatment discontinuation.⁹⁸
- Development of XDR-TB: The emergence of XDR-TB, resistant to second-line agents, such as fluoroquinolones, and at least one injectable agent, further limits therapeutic options, often requiring more toxic drugs such as bedaquiline and linezolid.²⁵
- Drug–drug interaction: The use of multiple drugs in MDR-TB therapy increases the risk of drug–drug interactions, for example, between bedaquiline and rifampicin (in cases of partial resistance), which may reduce efficacy or increase toxicity, thereby complicating regimen design.⁹⁷

- Delays in diagnosis: The slow growth rate of MTB (requiring weeks to months for culture-based diagnosis) delays drug resistance detection, leading to prolonged inappropriate therapy and poor clinical outcomes.¹⁰⁰
- Limited availability of advanced diagnostic tools: GeneXpert MTB/RIF, which rapidly detects rifampicin resistance, is not widely available in resource-limited settings due to cost and infrastructure requirements, contributing to underdiagnosis of MDR-TB.⁹⁸

False negatives: Diagnostic tests may fail to detect low-level resistance or resistance to drugs other than rifampicin, resulting in incomplete resistance profiles and suboptimal treatment regimens.¹⁰⁰

5.1.2. Nontuberculous mycobacteria infections

Therapeutic constraints, diagnostic hurdles, and mechanisms by which the pathogen evades the host immune system restrict treatment of NTM infections and contribute to long-term infection. Therapeutically and diagnostically, these include:

- Intrinsic drug resistance: The naturally occurring drug resistance in species such as *M. avium* and *M. abscessus* is characterized by a dense, lipid-containing cell wall and efflux pumps, making them resistant to most drugs, with complicated regimens typically involving macrolides, rifamycins, and aminoglycosides.³⁵
- Extended and toxic therapy: Therapy is generally 12–24 months long and includes agents such as amikacin, with a known risk for ototoxicity (auditory damage) and nephrotoxicity (nephropathy), with reduced patient compliance and high rates of failure.⁹⁹
- Variable drug susceptibility: NTM species exhibit varying susceptibility patterns; for example, macrolide resistance is commonly conferred by *M. abscessus* via the *Erm* gene, making regimens difficult to design and necessitating tests that are not standardized across all cases.⁴¹
- Drug–drug interactions: The risk is intensified when two or more drugs are administered simultaneously, as in clarithromycin and rifabutin, thereby lessening efficacy or increasing toxicity, thus making optimization difficult.³⁵
- Diagnostic delays: Differentiation from other mycobacteria is possible only through culture, which is slow (takes several weeks) and not commonly available, leading to delayed diagnosis and inappropriate initial therapy.³³
- Misdiagnosis hazards: Clinical presentations of NTM infection may resemble other lung diseases (e.g., bronchiectasis), and its non-specific biomarker profile

commonly results in misdiagnosis, especially outside specialist environments.⁹⁹

- Limited diagnostic availability: Whole-genome sequencing for species identification is expensive and out of reach in resource-poor environments, making accurate diagnosis difficult.³³

In addition, NTM employ immune evasion strategies that exacerbate therapeutic challenges: NTM prevents phagolysosomal fusion in macrophages through mechanisms such as LAM, thereby inhibiting bacterial killing.⁸⁴ It also reduces pro-inflammatory cytokines (e.g., TNF- α , IL-12) via inhibiting TLR signaling through LAM. It induces anti-inflammatory cytokines, such as IL-10, creating an immunosuppressive milieu that favors persistence.⁵⁰ Such techniques help NTM evade host death during therapy, leading to chronic infection.

5.2. Impacts of immune evasion on vaccine development

The impacts of immune evasion on vaccine development include:

- Delayed vaccine development: Vaccine development is hindered by antigenic diversity, immune suppression, and the lack of clear protective markers; however, subunit, whole-cell, and adjuvant techniques offer promising prospects.^{91,101–103}
- Antigenic variation and masking: MDR-MTB also changes its surface antigens, mycolic acids, and LAM, and hides them within a lipid cell wall containing phthiocerol dimycocerosates (PDIMs), making stable, immunogenic target antigens difficult to recognize.¹⁰¹ These variations and shields reduce antigen exposure to the host immune system, making vaccine design more difficult.¹⁰¹
- Suppression of immune response: MDR-MTB and NTM inhibit pro-inflammatory cytokines such as TNF- α and IL-12 by blocking TLR activation via molecules such as LAM and by inducing anti-inflammatory cytokines such as TGF- β and IL-10.¹⁰² The immunosuppression prevents the Th1-type immune response indispensable for containing mycobacterial infection, thereby impairing the efficacy of vaccines that depend on strong immune activation.
- Lack of correlates of protection: The complicated host–pathogen interactions (e.g., suppression of phagolysosomal fusion, modification of antigen presentation) hinder the identification of robust correlates of protection, such as antigen-specific T cells or titers.¹⁰³ Without unique markers, determining efficacy and the entire developmental process becomes complicated. Despite these difficulties, research

is advancing toward novel strategies to overcome immune evasion and provide immunological protection against MDR-MTB and NTM:

- Subunit vaccines: Antigen-specific vaccines, such as ESAT-6 and antigen 85B (Ag85B), have been shown to induce robust T cells, with the hope of inducing targeted immunity despite antigen variation.¹⁰³ The approach seeks to overcome some evasion methods by targeting conserved immunogenic sites.
- Whole-cell vaccine: These vaccines are prepared from attenuated or killed mycobacterial cells, such as BCG-derived strains or heat-killed *Mycobacterium vaccae*, and elicit widespread immune activation by presenting a diverse array of antigens.⁹¹ Such wide exposure is likely to combat specific immune-suppression methods of the illnesses.
- Developmental adjuvant: Vaccine efficacy is also augmented with new adjuvants, such as AS01 or CpG oligonucleotides, to enhance innate and adaptive immunity.¹⁰³ These adjuvants elicit significant Th1 responses that counteract the immunosuppressive effects of MDR-TB and NTM, thereby increasing the overall efficacy of vaccination.

In addition, a number of sophisticated vaccine platforms are at present being evaluated in clinical trials. Diverse possibilities for TB are being explored, such as M72/AS01E (protein–adjuvant, Phase IIb), VPM1002 (recombinant Bacillus Calmette–Guérin [BCG], Phase III), MTBVAC (live attenuated MTB, Phase II), and H56:IC31 (fusion protein + adjuvant, Phase II).^{98,104} Progress in NTM is relatively limited, with the most famous examples being DAR-901 (inactivated whole-cell *Mycobacterium obuense*, Phase II) and BCG revaccination trials.^{70,105} This comparison emphasizes that whereas TB vaccine development is moving across numerous platforms and

late-stage trials, NTM vaccines are rare, mostly in early-stage review, and reveal a translational gap between the two domains (Table 2).

5.3. Potential therapeutic targets based on immune evasion mechanisms

Nontuberculous mycobacteria and MDR-MTB have evolved immune evasion mechanisms that enable their persistence in the host, and targeting these immune-escape methods offers significant prospects for future treatment development. Strategies such as rapamycin, ROS/RNS blockers, cytokine agonists, biofilm and efflux pump inhibitors, and host-directed therapy (HDT) can antagonize immune evasion.^{20,21,40,42,106}

Several techniques to counter the evasion tactics of MDR-MTB are being explored:

- Induction of phagosome–lysosome fusion in macrophage compartments harboring MDR-MTB can enhance bacterial elimination. Autophagy stimulants such as rapamycin activate autophagy pathways, ensuring delivery to lytic compartments such as lysosomes for degradation, bypassing the pathogen's inhibition of this through proteins such as ESAT-6.²⁰
- Inhibitors of mycobacterial antioxidant enzymes, including superoxide dismutase and catalase, which degrade ROS and RNS generated by macrophages, are also targeted in MDR-MTB. For example, inhibition with agents such as thiourea derivatives can make MDR-MTB susceptible to host killing.¹⁰⁶
- Immunomodulatory strategies seek to counteract the inhibition of pro-inflammatory cytokine responses by MDR-MTB; agents such as IL-12 and IFN- γ agonists may reactivate Th1 immune responses by promoting activation of macrophages and T cells that are essential in controlling infection but are often suppressed

Table 2. Comparative clinical vaccine candidates for TB and NTM infections

Platform	TB candidates (Phase)	NTM infection candidates (Phase)
Protein/Adjuvant	M72/AS01E (IIb), H56:IC31 (II)	None (early MAC subunit preclinical)
Recombinant BCG	VPM1002 (III)	BCG revaccination (II)
Live attenuated	MTBVAC (II)	None
Wholecell inactivated	None	DAR901 (II)

Notes: This table summarizes advanced TB vaccine candidates currently in Phase II–III trials (M72/AS01E, VPM1002, MTBVAC, H56:IC31) alongside the limited NTM candidates under evaluation (DAR901, BCG revaccination). The comparison highlights the diversity and latestage progress of TB platforms versus the earlystage and fewer options available for NTM.

Abbreviations: BCG: Bacillus Calmette–Guérin; NTM: Nontuberculous mycobacteria; TB: Tuberculosis.

by mycobacterial components such as LAM.²¹ The development of these tailored medicines aims at precise immune evasion methods, offering prospects to improve outcomes against drug-resistant TB.

For NTM, therapeutic strategies also target immune evasion and intrinsic resistance to maximize therapeutic efficacy:

- Inhibition of biofilm formation, which is NTM's main evasion mechanism protecting it from antibiotics and immune responses. This initially involves using quorum-sensing inhibitors, such as furanone derivatives, or enzymes, such as DNase, to cleave the extracellular polymeric substances of the biofilm matrix, thereby facilitating better drug penetration and efficacy.⁴⁰
- Inhibition of efflux pumps can maximize antibiotic efficacy when treating intrinsic resistance in NTM. Efflux pumps such as RND-family efflux pumps remove antibiotics from bacterial cells, but inhibitors such as verapamil can maximize intracellular drug levels, thus bypassing resistance in species such as *M. abscessus*.⁴²
- Induction of drug-mediated autophagy in NTM-infected cells with medications such as metformin or vitamin D increases lysosomal bacterial breakdown by enhancing autophagosome-lysosome membrane fusion²⁰, which NTM inhibits with factors such as LAM.

Therapeutic targets shared by both MDR-MTB and NTM include HDTs such as cytokine therapy with IFN- γ or IL-12 to augment pro-inflammatory responses, and metabolic modulators targeting host pathways, such as cholesterol metabolism, to starve mycobacteria for survival nutrients, such as inhibiting cholesterol intake with statins.^{21,107} Moreover, combined therapies involving a convergence of antibiotics with immune-modulating drugs or HDTs improve outcomes by simultaneously targeting bacterial resistance and immune evasion, proposing a synergy-based strategy to deal with such difficult ones.¹⁰³

The clinical status of these therapies remains exploratory. Early-phase studies have shown potential for autophagy modulators, such as rapamycin and metformin, but have raised concerns about safety, including immunosuppression and metabolic side effects.¹⁰⁸ HDTs, such as IFN- γ adjunct therapy and checkpoint inhibitors (anti-programmed cell death protein 1 [PD-1]/programmed death-ligand 1 [PD-L1]), demonstrate potential, but efficacy data in patients with TB/NTM infections are preliminary and inconsistent.¹⁰⁹ Checkpoint inhibitors also carry risks of immune-related adverse events, such as autoimmunity and systemic inflammation,

which complicate their translational use in infectious disease settings.^{103,109,110} Biofilm and efflux pump inhibitors are largely preclinical, with translational challenges in drug delivery and toxicity. Overall, these tactics are promising, but their clinical implementation is hampered by safety concerns, uneven efficacy, and a lack of validated indicators to guide medication.

Future advancement will rely on incorporating these techniques into well-designed phase II/III trials with standardized outcomes and on validating the biomarker to ensure both efficacy and safety across varied patient populations.¹⁰⁹

6. Future directions

The investigation of immune evasion strategies by MDR-MTB and NTM has proceeded apace. However, considerable gaps remain in understanding the complex host-pathogen interactions that enable these microorganisms to persist and establish long-term infections. Filling these gaps will improve the tactics for controlling MDR-TB and NTM infections and will benefit global health outcomes.

Future research priorities include:

- Processes of immune modulation: Although much has been learned about the effects of MDR-MTB and NTM on the host immune response, the molecular mechanisms underlying these effects remain poorly understood. For example, the involvement of mycobacterial proteins, such as ESAT-6, and lipids, such as LAM, in downregulating pro-inflammatory cytokines (e.g., TNF- α , IL-12) or triggering anti-inflammatory responses (e.g., IL-10, TGF- β) warrants further investigation to identify novel therapeutic targets.¹⁰²
- Host genetic variation: Genetic host factors, such as polymorphisms in genes of immunological loci (e.g., *HLA*, *NRAMP1*), are important in determining susceptibility to mycobacterial illnesses. It is not apparent how they interact with pathogen immune evasion. Identification of genetic variations that influence immune responses to MDR-MTB and NTM may enable tailored therapeutic options.¹⁰³
- Biofilm production and persistence: Biofilm formation is an established immune-evasion mechanism in NTM, most notably in species such as *M. avium*. Its significance in MDR-TB remains unclear. Further investigations are needed to determine whether MDR-MTB forms biofilms in vivo (e.g., within lung cavities) and the role of biofilms in drug resistance and immune evasion by sheltering bacteria from immune cells and antibiotics.⁴⁰
- Latency mechanisms: The mechanisms by which

MDR-MTB and NTM establish latent infection, such as metabolic dormancy and immunological evasion, are poorly defined. Understanding how these infections evade immune identification during dormancy may inform ways to prevent reactivation, particularly in immunocompromised patients.⁹¹

- Impact on co-infections: Co-infection with other agents, such as HIV or respiratory viruses (e.g., influenza), may compromise host immune responses, thereby facilitating mycobacterial immune evasion through decreased T-cell function or cytokine production. Studies are needed to elucidate the effect of such co-infections on disease progression and management in MDR-TB and NTM infections.²⁵

6.1. Promising strategies

To address the obstacles, several approaches are being developed to control MDR-TB and NTM infections, with a focus on immune evasion and the latest computational techniques. Some of the promising strategies are:

- Host-directed therapies: HDTs include host response enhancement. Some examples include cytokines, such as IFN- γ or IL-12, which augment Th1-driven immunity; metabolic modulators (targeting host cholesterol metabolism) that restrict mycobacteria's nutrient supply (statins that block cholesterol feeding); and autophagy inducers, such as rapamycin and metformin, which improve bacterial clearance within cells by enhancing phagosome-lysosome fusion.^{20,21,107}
- Exploitation of immune evasion strategies: Drug design targeting specific evasion tactics will improve treatment outcomes. For example, phagosome-lysosome fusion restorers, efflux pump inhibitors (e.g., verapamil), and biofilm-disrupting agents (e.g., DNase or quorum-sensing inhibitors) may enhance antimicrobial efficacy. Efflux pump inhibitors increase intracellular antibiotic concentrations, whereas biofilm-disrupting agents improve antibiotic penetration and facilitate immune cell access to NTM.^{11,42,111}
- Novel vaccine strategies: Advancements in vaccine technology remain crucial, with innovative strategies such as subunit vaccines aiming at antigens like ESAT-6 and Ag85B to activate protective T-cell responses. Whole-cell vaccines offer broader immune engagement, including those based on attenuated or inactivated mycobacteria, such as BCG-derived strains. Meanwhile, messenger RNA (mRNA) vaccines use rapid-response platforms to simultaneously target multiple antigens.^{91,103,112}

6.2. Future research directions

Future research should focus on immune modulation, host genetics, biofilm dynamics, latency, and co-infections using HDTs, next-generation vaccines, precision medicine, artificial intelligence (AI), and a One Health framework.^{11,21,25,40,91,102,103,112,113}

Precision medicine improves treatment results by customizing therapy to an individual's genetic makeup and immune system. Genetic screening can help identify patients most likely to benefit from cytokine therapy or host-directed treatments.¹⁰³ AI and machine learning are accelerating medication and vaccine research through molecular target prediction, combination therapy optimization, and clinical data analysis to rapidly discover beneficial treatments.¹¹³ Finally, the One Health concept is becoming increasingly important, particularly given the environmental reservoirs of NTM (e.g., water systems) and the zoonotic potential of some species. Collaborative efforts across the human, animal, and environmental health sectors can help identify environmental sources and mitigate the risk of transmission.¹¹

6.3. Knowledge gaps

There has been progress, but important gaps remain:

- Host genetics: Polymorphisms of cytokine receptor, inflammasome, and autophagy genes are not well studied.
- Therapeutic targets: Efflux pump inhibitors, biofilm disruptors, and host deubiquitinase modulators (e.g., USP10, CYLD) are attractive but unproven candidates.
- Clinical translation: Genetic screening and AI-driven techniques are poorly integrated into practice.

Closing these gaps will improve clinical usefulness, accelerate therapeutic development, and provide the basis for next-generation vaccines and HDTs.

7. Conclusion

This review focuses on the diverse immune evasion tactics employed by MDR-MTB and NTM, including blocking phagosome-lysosome fusion, modulating cytokine responses, generating biofilms, and interfering with host autophagy and ubiquitination pathways. These mechanisms complicate and delay therapy and hinder vaccine development.

Novel therapies, such as autophagy inducers, HDTs, and innovative vaccination platforms, including mRNA-based approaches, may offer solutions to address these difficulties. However, there are significant knowledge gaps, particularly regarding host genetic variation, the

role of biofilm dynamics in MDR-TB, and the impact of co-infections on immune evasion.

Precision medicine approaches, such as genetic screening to customize treatments to an individual's immunological profile, must be included in future research, while AI-driven discovery platforms may accelerate drug and vaccine development. Further enhancement of therapy efficacy may be achieved by expanding therapeutic targets to include efflux pump inhibitors, biofilm disruptors, and modulators of host deubiquitinases. Finally, a One Health approach is needed to address environmental reservoirs of NTM and reduce transmission risk.

Integrating host genetics, AI, and novel therapeutic strategies into current interventions has the potential to transform the management of MDR-TB and NTM infections, advancing precision medicine and more effective vaccine development.

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Conflict of interest

The authors declare they have no competing interests.

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