Virulence Mechanisms of Mycobacterium abscessus: Current Knowledge and Implications for Vaccine Design

Kia C. Ferrell1,2,*, Matt D. Johansen3,4, James A. Triccas1,5 and Claudio Counoupas1,2,5,*

1 School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia,
2 Tuberculosis Research Program, Centenary Institute, Sydney, NSW, Australia, 3 Centre for Inflammation, Centenary Institute, University of Technology, Sydney, NSW, Australia, 4 Faculty of Science, School of Life Sciences, University of Technology, Sydney, NSW, Australia, 5 Sydney Institute for Infectious Diseases and the Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia

Mycobacterium abscessus is a member of the non-tuberculous mycobacteria (NTM) group, responsible for chronic infections in individuals with cystic fibrosis (CF) or those otherwise immunocompromised. While viewed traditionally as an opportunistic pathogen, increasing research into M. abscessus in recent years has highlighted its continued evolution into a true pathogen. This is demonstrated through an extensive collection of virulence factors (VFs) possessed by this organism which facilitate survival within the host, particularly in the harsh environment of the CF lung. These include VFs resembling those of other Mycobacteria, and non-mycobacterial VFs, both of which make a notable contribution in shaping M. abscessus interaction with the host. Mycobacterium abscessus continued acquisition of VFs is cause for concern and highlights the need for novel vaccination strategies to combat this pathogen. An effective M. abscessus vaccine must be suitably designed for target populations (i.e., individuals with CF) and incorporate current knowledge on immune correlates of protection against M. abscessus infection. Vaccination strategies must also build upon lessons learned from ongoing efforts to develop novel vaccines for other pathogens, particularly Mycobacterium tuberculosis (M. tb); decades of research into M. tb has provided insight into unconventional and innovative vaccine approaches that may be applied to M. abscessus. Continued research into M. abscessus pathogenesis will be critical for the future development of safe and effective vaccines and therapeutics to reduce global incidence of this emerging pathogen.

Keywords: Mycobacterium abscessus, cystic fibrosis, vaccines, virulence factors, reverse vaccinology

INTRODUCTION

The Mycobacterium abscessus clade are an emerging group of prominent human pathogens. Comprised of the three subspecies M. abscessus subsp. abscessus, M. abscessus subsp. bolletti, and M. abscessus subsp. massiliense and previously including closely related species Mycobacterium chelonae, these organisms are responsible for severe skin, soft tissue, and pulmonary infections
The M. abscessus clade is particularly problematic for individuals with enhanced susceptibility to pulmonary infection, such as those with bronchiectasis or chronic obstructive pulmonary disease (COPD), prior tuberculosis (TB) infection, or individuals with cystic fibrosis (CF; Griffith et al., 2007). The incidence of NTM infection in individuals with non-CF bronchiectasis is particularly high, with an estimated 37% of all patients with this condition having an NTM infection (Mirsaeidi et al., 2013). CF patients are particularly susceptible to NTM infection, with incidence rates in one study reported to be 1,000 times higher than in the general population (Martiniano et al., 2019). CF is a recessive genetic disorder affecting the cystic fibrosis transmembrane conductance receptor (CFTR) and resulting in dysregulated chloride transport, with a wide affect across different organs including the lung. Individuals with CF have dysregulated mucus production and are particularly susceptible to bacterial infections that become chronic and difficult to eradicate (Cantin et al., 2015). Mycobacterium abscessus infection is extremely difficult to treat due to intrinsic, adaptive, and acquired antibiotic resistance traits; these result in poor treatment success rates as low as 30% depending on the subspecies (Nessar et al., 2012; Koh et al., 2014). Furthermore, the establishment of chronic M. abscessus infection is associated with a significant decline in lung function, which has a profound impact on patient quality of life (Esther et al., 2010; Kwak et al., 2019). Despite the significant impact of M. abscessus infection on susceptible populations, there is currently no vaccine available (approved or within clinical trials) for M. abscessus. Given both the difficulty in treating M. abscessus and the poor prognosis following the establishment of persistent and chronic infection, there is an urgent need to develop novel prophylactic interventions to reduce incidence of M. abscessus infections in at-risk populations.

The development of novel vaccines for pathogens, such as M. abscessus, can be guided by the characterization of novel virulence factors (VFs), molecular components which facilitate pathogen survival and persistence in the host. Bacterial VFs promote pathogen resistance to immune defenses, improve adherence or invasion of host cells/tissues or enhance survival through modification of the host environment (Wu et al., 2008). The study of virulence determinants can identify targets for the attenuation of pathogens, or proteins which themselves may be sufficiently immunogenic to be formulated into a vaccine (Ottenhoff and Kaufmann, 2012). For example, M. tuberculosis antigen 85 complex proteins are VFs that promote bacterial entry into host cells; the immunogenic nature of these proteins has led to their inclusion in numerous recombinant, subunit, and viral vectored vaccines (Babaki et al., 2017). Although some VFs are unsuitable for incorporation into vaccines—due to poor immunogenicity or unsuitable cellular location for immune exposure—mechanistic studies of these VFs can shed light onto host responses to infection and thus informs future vaccine studies.

While traditionally viewed as an opportunistic pathogen, there is now compelling evidence that the M. abscessus clade possesses hallmark characteristics of a true pathogen. Work on M. abscessus in recent years has shed light on the unique disease pathogenesis and VFs possessed by this clade. While M. abscessus has multiple well-characterized mycobacterial VFs, it also possesses non-mycobacterial VFs which share high homology with other notable CF pathogens (Ripoll et al., 2009). Given the developments in our understanding of M. abscessus virulence in recent years, the purpose of this review is to summarize our current knowledge on M. abscessus VFs, as well as recent research efforts to develop vaccines against this emerging pathogen. An overview of M. abscessus biology and pathogenesis in the context of non-tuberculous mycobacteria (NTM) can be found in Johansen, Herrmann (Johansen et al., 2020).

**EVOLUTION OF MYCOBACTERIUM ABSCESSUS INTO A HUMAN PATHOGEN**

Many species of NTM are ubiquitous in both urban and natural environments. NTM are adept at survival in numerous habitats, such as soil, peats, and swamps, where the thick hydrophobic membrane of Mycobacterium spp. facilitates strong adherence to different surfaces and likely promotes survival in hostile environments (Falkinham, 2009). This resilience to different environmental conditions has allowed the permeation of NTM into urban settings, where species, such as M. abscessus, are isolated from potable water sources and plumbing systems (Thomson et al., 2013). Hospital outbreaks of NTM infections are frequently linked to contaminated water supplies (Tam et al., 2014; Guimarães et al., 2016); acquisition of infection from environmental sources, rather than from other infected individuals, is through to be the major transmission route by which M. abscessus gains entry to human hosts (Thomson et al., 2013). The prevalence of M. abscessus in nosocomial environments, combined with high-level resistance to many glutaraldehyde-based hospital-grade disinfectants (Burgess et al., 2017), has facilitated the establishment of opportunistic M. abscessus infections in both immunocompetent and immunocompromised individuals.

Given the incursion of M. abscessus into urban environments, such as plumbing systems and water sources, the occurrence of opportunistic NTM infections is unsurprising. However, the diverse array of VFs possessed by the M. abscessus clade points to a more complex evolution of this organism into a human pathogen. Importantly, NTM including M. abscessus have been isolated from free-living amoeba derived from urban water sources, suggesting a role for amoeba in enabling NTM persistence (Delafont et al., 2014). It has been proposed that early adaptations of M. abscessus to an intracellular amoeba lifestyle may have enhanced M. abscessus virulence and promoted survival in mammalian hosts (Thomas and McDonnell, 2007). Preculture of M. abscessus in Acanthamoeba castellanii amoeba enhances persistence in vivo in a murine model of infection (N’Goma et al., 2015). Mycobacterium abscessus also remain viable when encysted by amoeba, suggestive of both their adaptation to the intracellular lifestyle and the potential for amoeba to act as an environmental
reservoir for *M. abscessus* (da Silva et al., 2018). Dubois et al. (2019) performed extensive transcriptomic analysis to track changes in gene expression induced in *M. abscessus* during intracellular growth. It was demonstrated that growth of *M. abscessus* in amoeba and macrophages induced similar patterns of differential gene expression, including an upregulation of genes to cope with intracellular stresses, such as heat shock and oxidative stress (e.g., *GroEL-ES* and *hsp*), a switch to slower growth phenotype, and utilization of fatty acids as an energy source. Following selection of *M. abscessus* genes upregulated in amoeba and subsequent expression of these genes in opportunistic pathogen *M. chelonae*, the authors showed enhanced growth of complemented *M. chelonae* in macrophages. This supports the theory that growth of *M. abscessus* in amoeba facilitates enhanced infection and survival in mammalian macrophages, and also highlights the diverse range of mechanisms *M. abscessus* uses to persist in the host (Dubois et al., 2019).

Although most NTM infections are acquired from environmental sources, phylogenetic analysis of *M. abscessus* clinical isolates suggests that CF centers may facilitate indirect person-to-person transmission of *M. abscessus* clones through fomites (Aitken et al., 2012). Whole genome sequencing of *M. abscessus* isolated in global CF centers has revealed the presence of dominant *M. abscessus* clones with diverse geographical distribution. In addition to comprising 70% of total global *M. abscessus* infections, these dominant isolates display heightened virulence in macrophages and in Severe Combined Immunodeficiency (SCID) mice (Bryant et al., 2016). This is reflective of trends observed with other well-characterized CF pathogens, such as *Pseudomonas aeruginosa*, where epidemic strains are associated with worse patient outcome (Panagea et al., 2003; Fothergill et al., 2012).

While patterns of evolution in CF pathogens have been extensively described for other species, such as *P. aeruginosa*, only recently have similar patterns been described in *M. abscessus*. Bryant et al. recently used single nucleotide polymorphism analysis of global *M. abscessus* clinical isolates to define the trajectory of *M. abscessus* evolution into a true pathogen (Bryant et al., 2021). The initial horizontal acquisition of genes from unrelated species (as described by Ripoll et al., 2009) resulted in a significant leap in *M. abscessus* genomic variation. Following this, the transition of *M. abscessus* to the preferred infection site of the lung and evolution into a pulmonary pathogen was coupled with a further increase in *M. abscessus* genomic variation. This is unsurprising given the crucible of selective pressures present within the CF lung, such as antibiotic stressors, interspecific competition, and host immune defenses all encouraging the dominance of favorable traits (Harrison, 2007; Folkesson et al., 2012). Furthermore, the physical separation of different lung lobes coupled with the heterogeneity of CF lung tissue creates numerous ecological niches that facilitate species diversification (Harrison, 2007). Bryant et al. confirmed this hypothesis with the identification of numerous, genetically distinct subclones of *M. abscessus* isolated from different areas of the lung in individual patients (Bryant et al., 2021). The authors also described the presence of *M. abscessus* clones with hypermutable phenotypes, capable of greater phenotypic variation likely due to DNA damage from the highly oxidative pulmonary environment (Ciofu et al., 2005; Martina et al., 2014). While these results identified strong evolutionary pressure on *M. abscessus* genes that promote intramacrophage survival, perhaps the most prominent finding was the apparent fitness cost of enhanced virulence of *M. abscessus* isolates. Importantly, mutants with virulence mutations display reduced transmission rates, presumably due to their impaired survival on fomites. As such, the “evolutionary potential” or continued gain of virulence of *M. abscessus* is limited provided direct patient-to-patient spread (through aerosolized droplets) does not occur (Doyle et al., 2020; Bryant et al., 2021). However, whether *M. abscessus* can spread through aerosols is still a matter that requires clarification (Bryant et al., 2013). Given the demonstrated potential of this species to acquire polymorphisms that promote *M. abscessus* survival and the possibility for continued species adaptation, there is a significant need for the continued identification of *M. abscessus* VFs.

**MYCOBACTERIA-SPECIFIC VIRULENCE FACTORS**

The genus *Mycobacterium* contains approximately 200 species, many of which inhabit soil or water environments and have occasional interaction with humans (Tortoli et al., 2017; Armstrong and Parrish, 2021). However, some members of this genus, such as *M. tb* and *Mycobacterium leprae*, are extremely successful human pathogens, being the causative agents of TB and leprosy, respectively (Gupta et al., 2018). Similarly, *Mycobacterium marinum* and *Mycobacterium ulcerans* are NTM infections acquired by environmental exposure but are adept at establishing cutaneous infection in humans (Tan et al., 2020). The success of these species is tied closely with their ability to survive within the host using an extensive collection of Mycobacteria-specific VFs. As our understanding of *M. abscessus* virulence expands, there is continual discovery of virulence traits resembling other Mycobacterial species. These further contribute to the definition of *M. abscessus* as a truly pathogenic species.

**Mycobacterial Membrane Protein Large Proteins**

Mycobacterial membrane protein large (MmpL) proteins are a family of VFs of the *Mycobacterium* genus, responsible for transport of lipids and siderophores to the periplasmic space (Melly and Purdy, 2019). These include complex lipids that are essential for the integrity of the mycobacterial envelope, which forms a barrier of protection from immune cells and chemotherapeutic agents (Bailo et al., 2015). Many of these lipids play prominent roles in modifying host–pathogen interactions, and as a result mutations within MmpL proteins can lead to alterations in mycobacterial virulence (Chalut, 2016).
The MmpL class of transport proteins is overrepresented in *M. abscessus*, with 31 identified MmpL proteins in the reference strain compared to 14 within *M. tb* (Deshayes et al., 2010). MmpL4 proteins have an important contribution to *M. abscessus* virulence through their transport of glycopeptidolipids (GPL) to the outer mycobacterial surface which gives colonies a glossy and smooth appearance on solid agar. Disruption of MmpL4 proteins results in the conversion from the smooth morphotype to a rough cording morphotype, the latter of which is associated with enhanced virulence due to large serpentine cord formation, triggering pro-inflammatory responses and apoptosis (Pawlik et al., 2013). Disruption of *M. abscessus* MmpL8 reduces the transport of glycosyl-diacylated-nondecyl-diols (GDND), a novel glycolipid that facilitates bacterial adhesion to macrophages and induces macrophage phagosomal rupture, and thereby reduces mycobacterial virulence (Halloum et al., 2016). MmpL proteins from mycobacterial species, such as *Mycobacterium smegmatis*, also confer resistance to some antibiotics, such as isoniazid, by acting as an efflux pump (Pasca et al., 2005; Viljoen et al., 2017). Mutations within transcriptional regulator *tetR*, which controls two MmpS-MmpL gene pairs, promote resistance to clofazimine and bedaquiline in *M. abscessus* (Richard et al., 2019), while MmpS5-MmpL5 are associated with efflux of thiacetazone derivatives (Halloum et al., 2017). Absence of either MmpL-P pair increases susceptibility of intracellular *M. abscessus* to bedaquiline, indicating the contribution of this protein to providing antibiotic resistance (Gutiérrez et al., 2019). Given the role of MmpL proteins in enhancing or attenuating virulence and conferring antimicrobial resistance, this a particularly noteworthy area of continued research for the development of *M. abscessus* vaccine candidates.

**ESX Secretion Systems**

Members of the *Mycobacterium* genus possess an outer mycomembrane comprised of components that must be transported across the cell membrane to reach the extracellular space. Early secretory antigenic target (ESAT-6) secretion (ESX) systems, also known as Type VII secretion systems, are important players in facilitating transport of proteins across this outer membrane and are known to play a significant role in mycobacterial virulence, nutrient acquisition, and bacterial conjuction (Lagune et al., 2021). There are five characterized ESX secretion systems spread across the *Mycobacterium* genus, varying in complexity and in function (Gröschel et al., 2016). However, all ESX systems have a conserved set of genes that include the machinery for substrate secretion, accessory proteins, and ESX secreted proteins (Houben et al., 2014). The most extensively studied ESX system, ESX-1, facilitates *M. tb* intracellular survival via inhibition of phagosomal acidification and induces phagosome rupture and mycobacterial escape to the cytosol (Sturgill-Koszycki et al., 1994; van der Wel et al., 2007). The contribution of other ESX secretion systems to virulence is less well-characterized; however, the ESX-3 system of *M. tb* is known to be utilized for iron acquisition in low nutrient environments and the absence of this system attenuates growth in vivo (Siegrist et al., 2009; Tufariello et al., 2016). The role of the ESX-5 secretion system in slow-growing mycobacteria relates to the integrity of the mycobacterial capsule, with disruption of ESX-5 in *M. marinum* leading to inability of this pathogen to disrupt the cellular membrane (Ates et al., 2016). While attenuation of ESX-5 deficient *M. marinum* is evident in zebrafish embryos, the same mutant is hypervirulent in adult zebrafish (Weerenburg et al., 2012). Given that zebrafish embryos solely possess innate immunity, while the adult zebrafish harbors both innate and adaptive immunity, these findings suggest that ESX-5 may play different roles in disease pathogenesis depending on whether innate or adaptive immunity is the major driver of cellular control of mycobacterial infection (Cronan and Tobin, 2014).

There are currently three characterized secretion systems of *M. abscessus*—ESX-3, ESX-4, and ESX-P. Secretion targets of ESX-3 (ExG/H proteins) induce pro-inflammatory cytokine production when co-cultured with bone marrow-derived macrophages, and mice infected with ESX-3-deleted *M. abscessus* display a reduced inflammatory and granulomatous response coupled with decreased bacterial survival (Kim et al., 2017). However, this work did not elucidate specific mechanisms by which ESX-3 promotes *M. abscessus* growth in vivo. Furthermore, this study did not include complementation of ESX-3 function in *M. abscessus*, highlighting the need for further investigation into the role of ESX-3 in *M. abscessus* virulence. In contrast, the function of ESX-4 within *M. abscessus* was recently characterized in detail (Laencina et al., 2018). Through generation of an extensive mutagenesis library, Laencina et al. associated disruptions of the ESX-4 locus with a decrease in intramacrophage and amoeba survival. ESX-4 associated ATPase EccB4 was shown to contribute to virulence by limiting phagosomal acidification and facilitating cytosol contact through phagosomal membrane damage. Unlike ESX-3 and ESX-4, ESX-P is a plasmid-borne secretion system unique to a clinical isolate of *M. bolletti* (Dumas et al., 2016). More recent work has characterized plasmid-borne ESX components within *M. abscessus* clinical isolates, suggesting a more extensive presence of ESX systems in this species than previously thought (Dedrick et al., 2021). Whether these secretion systems are functional and contribute to *M. abscessus* virulence is an area of continued research, and may represent an unexplored means by which *M. abscessus* is able to acquire novel VFs that contribute to evolving pathogenicity.

**Lsr2**

Lsr2 is a histone-like protein possessed by several members of the *Mycobacterium* genus, with roles in transcriptional regulation and DNA damage protection. In *M. tb*, Lsr2 is required for normal growth in anaerobic and DNA damage-inducing conditions (Bartek et al., 2014). When functioning as a transcriptional regulator, the binding of Lsr2 to coding sequences (of which VF genes are common) may contribute to the transition to a latent metabolic state that occurs during chronic *M. tb* infection (Gordon et al., 2010; Bartek et al., 2014). In *M. smegmatis*, lsr2-knockout strains have decreased persistence within macrophages, an effect which is reversed following scavenger treatment to sequester free radicals (Colangeli et al., 2009). *Mycobacterium smegmatis* lsr2 knockouts also
display a smooth, glossy phenotype and are defective in biofilm and pellicle formation (Chen et al., 2006). This is reflected in *M. abscessus*, where rough morphotypes display greater levels of Lsr2 expression than smooth morphotypes. While *M. abscessus* *lsr2* knockouts are not affected in their glycopeptidolipids (GPL) profile, *lsr2* knockout in the rough morphotype of *M. abscessus* show greater susceptibility to reactive oxygen intermediates and display reduced survival within macrophages, as well as in the zebrafish and murine model of infection, particularly at later time points during infection (Le Moigne et al., 2019). Given the importance of this transcriptional regulator for pathogenesis of *M. abscessus*, Lsr2 may be a promising target for drug development (Liu and Gordon, 2012).

**Glycopeptidolipids**

*Mycobacterium abscessus* grown on solid agar medium displays distinct colony morphologies characterized as smooth (S) and rough (R) morphotypes, each with unique patterns of virulence. These are linked to the variable expression of GPL on the cell surface of *M. abscessus*, with expression of GPL within S morphotypes resulting in round, glossy colonies, while absence of GPL leads to R morphotypes resulting in a dry, corded appearance (Nessar et al., 2011). The localization of *M. abscessus* GPL has been shown to cluster to specific nanodomains on the bacterial surface, ultimately modulating surface hydrophobicity and likely influencing bacterial adhesion and virulence (Nessar et al., 2011; Vlijmoen et al., 2020). The conversion of S to R occur as a result of mutation within genes located within the GPL locus, often associated with GPL synthesis or secretion (Nessar et al., 2011; Pawlik et al., 2013; Park et al., 2015; Li et al., 2020). To date, the mechanisms responsible for S to R conversion are not known; however, this has been hypothesized to occur in response to stress (e.g., Antibiotic stress) which may manifest during host colonization (Bernut et al., 2016b; Gutiérrez et al., 2018). R morphotypes are more frequently isolated from CF patients with chronic infection, and display heightened virulence compared to smooth morphotypes (Jönsson et al., 2007; Kreutzfeldt et al., 2013). The differences in virulence can be linked to different interactions between S and R morphotypes with immune subsets following infection.

As surface molecules on *M. abscessus*, the absence of GPL on the surface of R morphotypes alters the initial interaction between bacteria and phagocytic cells. A lack of surface GPL exposes immunostimulatory ligands such phosphatidylinositol mannoside (PIM) moieties which activate Toll-like receptor (TLR) signaling, resulting in downstream pro-inflammatory cytokine production (Rhoades et al., 2009). While S morphotypes also express PIMs, the heightened expression of surface GPL masks these TLR ligands which acts to prevent innate cell activation (Davidson et al., 2011). Polar GPL themselves also act in an immunosuppressive manner, dampening pro-apoptotic signals induced by R morphotypes and limiting reactive oxygen species (ROS) production (Whang et al., 2017). It is likely that GPL also play an important role in cell–cell interactions, with post-translational modifications of GPL affecting adherence and invasion of *M. abscessus* to macrophages (Daheer et al., 2020). Remodeling of surface GPL also occurs following growth in artificial CF sputum, although the biological significance of this is unknown (Wiersma et al., 2020). Together, these data point to the prominent role of GPL in defining early infection outcomes, with immunologically “silent” establishment of S morphotype *M. abscessus* in the upper respiratory tract likely facilitating initial colonization of the lung (Gutiérrez et al., 2018).

The S and R morphologies of *M. abscessus* interact differently with immune cells, leading to different patterns of growth in *vivo*. While S morphotypes exist largely as single bacilli, R morphotypes form clumps which are difficult for phagocytic cells to engulf (Roux et al., 2016). These aggregates remain adhered to phagocytic cups or, if small enough, are contained within “social” phagosomes containing multiple bacilli. Within the macrophage, R morphotypes appear resistant to lysosomal degradation, leading to extensive and rapid intracellular growth, followed by macrophage apoptosis (Aguilo et al., 2013; Kim et al., 2019). Following apoptosis, rough bacilli are then released into the extracellular space whereby they replicate freely and aggregate to form large extracellular serpentine cords which resist phagocytosis, causing excessive inflammation and abscess formation (Bernut et al., 2014a). Disruption of *M. abscessus* cording attenuates virulence *in vivo*, indicating the importance of this phenotype in dictating the outcome of infection (Bernut et al., 2014a; Halloum et al., 2016).

S morphotypes of *M. abscessus* also resist intracellular degradation, through more direct manipulation of macrophage effector function. Phagocytosed S bacilli often exist in “lone phagosomes,” and act to limit phagosomal acidification and disrupt phagosome membrane integrity, which may facilitate escape to the cytosol (Roux et al., 2016). While cytosol escape and blocking of phagosome acidification are associated with functional ESX-4 found in *M. abscessus*, it is unclear what role surface GPL may play in facilitating this effector function (Gutiérrez et al., 2018; Laencina et al., 2018). Given the integral link between mycobacterial virulence and macrophage function, understanding the mechanisms by which *M. abscessus* interacts with innate immune cells is critical in our understanding of *M. abscessus* virulence (Feng et al., 2020).

**Other Cell Surface Molecules**

The structure of the mycobacterial cell wall and outer envelope is comprised of a complex array of lipoproteins, glycolipids, and glycoproteins, many of which take part in host–pathogen interactions (Jackson, 2014). These may directly modulate immune responses, or improve the structural integrity of the mycomembrane which renders *M. abscessus* more resistant to immune mediators (Karakuosis et al., 2004). For example, succinylation of *M. abscessus* surface polysaccharides alters intracellular survival by an uncharacterized mechanism, possibly in relation to altered cell surface hydrophobicity and charge (Paličková et al., 2020). Other VFs may arise from proteins associated with the modification or transport of cell surface molecules. Knockout of *pmt*, a protein-O-mannosyltransferase which is responsible for glycosylation of lipoproteins in the mycobacterial cell envelope, increases cell wall permeability...
which may enhance susceptibility to innate cellular defenses, such as ROS (Becker et al., 2017). Similarly, probable N-acetyl
transferase Eis2 is thought to play a role in cell wall biogenesis or transport of cell wall components; deletion of this VF
increases M. abscessus susceptibility to ROS and H₂O₂ and reduces intracellular survival (Dubois et al., 2019). Expression
of some surface components, such as trehalose polyphosphates (TPPs), does not directly affect macrophage viability, but promote
virulence by facilitating M. abscessus cording (Llorens-Fons et al., 2017). Mycobacterium abscessus cell envelope-derived
lipids, such as cardiolipin or I-mannosides, are also effective at neutralizing LL-37, an antimicrobial peptide produced by
neutrophils and macrophages (Honda et al., 2015, 2020). It has been established that different growth conditions alters
the cell wall composition of M. abscessus; whether this occurs in an infection setting and how this impacts on the outcome
of infection remains to be established (Hunt-Serracin et al., 2019).

NON-MYCOBACTERIA-SPECIFIC
VIRULENCE FACTORS

While M. abscessus was first isolated in 1952, complete sequencing
of the M. abscessus genome was completed relatively recently
in 2009 (Brown-Elliott and Wallace, 2002; Ripoll et al., 2009).
In their genomic analysis of M. abscessus, Ripoll et al. identified
regions syntenic with non-mycobacterial species, including
Actinobacteria, such as Rhodococcus, as well as opportunistic
CF pathogens P. aeruginosa and Burkholderia cepacia (Ripoll
et al., 2009; Mahenthiralingam, 2014). The organization of these
genes into large clusters was suggestive of en bloc horizontal
gene transfer events from distant species, with some of these
regions encoding proteins that are known to promote virulence
in other species. The functions of these VFs are well defined
in other species, with most facilitating survival in harsh or
stressful conditions. Some are involved in early P. aeruginosa
colonization and/or persistence in CF airways, such as phenazine
biosynthesis and homogentisate catabolism genes (Rodriguez-
Rojas et al., 2009; Jimenez et al., 2012). VFs involved in iron
acquisition and resistance to reactive nitrogen intermediates
(RNI) were also identified, with sequence homology to
Rhodococcus spp. (Ripoll et al., 2009). Similarly, the M. abscessus
genoce contained proteins with predicted function in
phenylacetic acid degradation; these promote B. cepacia
survival in the Caenorhabditis elegans models of infection,
although their precise mechanism of action is unknown (Law
et al., 2008; Loutet and Valvano, 2010). Some of these
non-mycobacterial VFs have been examined in detail (Figure 1),
however for the most part their function in M. abscessus
virulence is yet to be established.

Phospholipase C

Phospholipases are virulence determinants found in a diverse
range of mycobacterial and non-mycobacterial species, including
P. aeruginosa, Staphylococcus aureus, and Listeria monocytogenes
(Ghannoum, 2000). Phospholipases promote virulence by allowing
escape from the phagosome and cell-to-cell spread, which is
thought to be mediated by cleavage of phospholipids that form
part of the cell membrane (Camilli et al., 1991; Smith et al.,
1995; Ghannoum, 2000). Expression of plc genes by P. aeruginosa
is associated with phosphate limiting conditions, and disruption
of plcC results in a reduction of P. aeruginosa virulence in the
murine model of infection (Ostroff et al., 1989). Similarly,
phospholipase C is upregulated by virulent M. tb in phosphate
limiting conditions, such as those that may occur in an intracellular
environment (Le Chevalier et al., 2015). PlcC isolated from M.
abscessus induces macrophage lysis, and plcC knockout strains
display reduced survival within amoeba (NG'Goma et al., 2015).
Given the sequence similarity between plcC from M. abscessus
and other CF pathogens (NG'Goma et al., 2015), PlcC is a promising
antigenic target for vaccine development. CF patients with
pulmonary NTM infection or P. aeruginosa infection have higher
titeres of anti-PlcC antibodies in serum than uninfected individuals,
demonstrating immune exposure to the PlcC antigen following
infection (Le Moigne et al., 2015). Vaccination with M. abscessus
plcC DNA induces potent antibody responses and a moderate
reduction in bacterial burden in ΔF508 mice, which possess
the most common CF gene mutation (Cutting, 2015; Le Moigne
et al., 2015). However, the role of PlcC in nutrient acquisition
in the lung, and if it enables M. abscessus to maintain long-
term infection is unclear (Raynaud et al., 2002).

MgtC

MgtC plays an essential role in the survival of pathogens such
Salmonella enterica subsp. typhimurium and CF pathogens B.
cepacia and P. aeruginosa (Alix and Blanc-Potard, 2007; Belon
et al., 2015). While the physiological function of this protein
has not been fully elucidated, MgtC is a membrane protein that
confers survival benefits for both intracellular and extracellular
pathogens (Belon et al., 2015). Expression is essential for growth
in magnesium poor conditions for P. aeruginosa and B. cepacia,
while upregulation of MgtC following phagocytosis provides
enhanced intracellular survival and resistance to low pH for
intracellular pathogen Salmonella spp. (Maloney and Valvano,
2006; Rang et al., 2007; Lee and Lee, 2015). MgtC appears to
perform a similar role in M. tb, as the protein contributes to
improved survival in low magnesium and pH conditions and
enhanced virulence in mice (Buchmeier et al., 2000). Mycobacterium
abscessus has two sequences encoding proteins homologous to
MgtC, MAB_3953, and MAB_0146, both of which bear sufficient
sequence similarity to S. typhurrium MgtC, as they can partially
restore its function in an mgtC-deficient strain (Le Moigne et al.,
2016). Mycobacterium abscessus MgtC is induced in low Mg²⁺
conditions and within the macrophage, mgtC-encoding DNA
provides protection against M. abscessus challenge (Le Moigne
et al., 2015, 2016). Of note, M. abscessus lacking functional
MgtC does not show a significant impairment in macrophage
survival, which may indicate a role of this VF in the extracellular
stage of M. abscessus growth (Bernut et al., 2014a).

Porins

Porins are a diverse class of pore proteins that play an important
role in the transport of hydrophilic molecules into the bacterial cell.
Porins are expressed widely among gram-positive and negative virulence of different species (Achouak et al., 2001). The contribution of porins to the virulence of different species of pathogenic bacteria is extremely diverse and includes facilitating cell adherence, inducing host cell apoptosis and transporting surface proteins associated with virulence (Müller et al., 1999; Azghani et al., 2002; Brunson et al., 2019). Within the context of mycobacteria, M. smegmatis has a collection of pore-forming proteins that regulate nutrient influx which is important for maintaining normal growth rates (Stephan et al., 2005). Deletion of M. smegmatis porin MspA, MspC, and MspD improves M. smegmatis survival in macrophages, by providing greater resistance of M. smegmatis to host-produced nitric oxide (NO; Fabrino et al., 2009). Similarly, deletion of porins mmpA/B in M. abscessus also improves M. abscessus intracellular survival within phagocytic cells and bacterial persistence in SCID mice (de Moura et al., 2021). Porin knockout strain ΔmmpA displays reduced uptake of glucose, but mutants did not display varied uptake by macrophages, susceptibility to NO, or cell cytotoxicity. Of note, sequential samples taken from different early and late M. abscessus infections in CF patients show mutations within the mmp porin genes (Lewin et al., 2021). However, phylogenetic analysis by de Moura et al. could not establish a contributing role of porin mutations to enhanced virulence or transmission of dominant clinical M. abscessus isolates (de Moura et al., 2021). This suggests that while this class of proteins are significant VFs of other species, porins possessed by mycobacterial species including M. abscessus may perform a role unrelated to virulence.

**FIGURE 1** | Virulence factors of M. abscessus. M. abscessus uses an extensive range of virulence factors to facilitate survival within host cells. These include membrane bound proteins, pores and secretion systems (i); secreted proteins with a role in virulence (ii); transcriptional regulators and nucleic acid associated proteins (iii); proteins involved in the modification (iv) and transport (v) of cell surface components; and molecules that comprise the outer mycobacterial membrane (vi).

**TOWARD THE DEVELOPMENT OF A MYCOBACTERIUM ABSCESSUS VACCINE: POSSIBILITIES AND CHALLENGES**

The global incidence of NTM including M. abscessus is increasing, with disease prevalence within CF populations increasing from 9% to 13% in studies conducted after 2000 (Marras et al., 2007; Qvist et al., 2014, 2015; Bar-On et al., 2015; Adjemian et al., 2018). Chronic infection with M. abscessus leads to decreased lung function and is a significant contributor to morbidity and mortality of affected individuals (Esther et al., 2010; Qvist et al., 2015). This combined with the extensive antibiotic resistance and large repertoire of VFs of this pathogen...
(Figure 1; Table 1) emphasizes the need for prophylactic strategies to limit the global burden of M. abscessus. Given there is currently no vaccine available for M. abscessus and none in clinical development, there is a clear unmet need for the development of vaccines against this pathogen. However, development of a potential M. abscessus vaccine must consider target populations for vaccination, and specific challenges associated with immunizing these populations. This includes an understanding of immune correlates of protection against M. abscessus in both immunocompetent and immunocompromised individuals, in addition to gaining knowledge to efforts to develop a vaccine for M. tb.

Immune Responses to Mycobacterium abscessus Infection

A thorough understanding of the immune correlates of protection against M. abscessus infection is critical for the development of an effective M. abscessus vaccine. This is an area of ongoing research, primarily focused on the use of in vitro, zebrafish, and murine models (Bernut et al., 2015, 2017; Caverly et al., 2015; Meir et al., 2018). Incorporating these different approaches has led to a greater knowledge of immune mechanisms involved in protection against this pathogen.

As with other members of the Mycobacterium genus, early and robust innate immune responses appear to play a critical role in shaping immune control of M. abscessus infection. Monocytes and macrophages are the most common cell subset infected by M. abscessus in human lung tissue (Ganbat et al., 2016). Both S and R morphotypes of M. abscessus are rapidly phagocytosed by macrophages early after infection and depletion of this subset substantially increases bacterial burden in zebrafish (Bernut et al., 2014a, 2019). ROS and NO are major bactericidal mechanisms employed by macrophages to kill M. abscessus, with production of NO and ROS strongly correlated with a protective effect across in vitro and animal models of M. abscessus infection (Kim et al., 2014a; Lee et al., 2017). Interestingly, NO production is enhanced by a type 1 interferon (TI IFN) response and prophylactic administration of rIFN-B can promote bacterial clearance (Lee et al., 2017). This is contrary to recent work on M. abscessus suggesting that TI IFNs promote macrophage apoptosis and bacterial spread from cell-to-cell (Zhang et al., 2019). In humans, the role of NO and ROS in providing protection against M. abscessus is less clear. When used as an inhaled therapeutic, NO improves lung function and quality of life in CF patients, but this has not been definitively linked to reduction in M. abscessus burden (Bentur et al., 2020). An oxidative environment within the macrophage also appears to enhance M. abscessus growth in an ex vivo setting (Oberley-Deegan et al., 2010). Moreover, NOS2 knockout (Nos2−/−) mice do not display exacerbated bacterial burden compared to wild type (Obregón-Henao et al., 2015). Taken together, these findings suggest a redundant or possibly detrimental role of ROS in protection against M. abscessus infection.

As early responders to pulmonary infection, neutrophils are a prominent component of the immune response against M. abscessus. Neutrophil accumulation is driven by TNF and IL-8 production by macrophages and alveolar epithelial cells, and this subset congregates within murine lungs following infection with both S and R morphotypes (Caverly et al., 2015; Bernut et al., 2016a; Malcolm et al., 2018). Despite the integral role of neutrophils in the formation of the granuloma, the efficacy of this subset in reducing bacterial burden remains unclear. Neutrophils phagocytose bacteria and employ neutrophil extracellular traps (NETs) and ROS production to eliminate M. abscessus, but neutrophil-derived ROS and LL-37 has limited killing activity against M. abscessus (Honda et al., 2015; Malcolm et al., 2018). Extracellular DNA released in response to M. abscessus during NETosis may also contribute to biofilm formation in the CF lung (Malcolm et al., 2013). Pulmonary infection with the more virulent R morphotype of M. abscessus is also accompanied by neutrophil accumulation in the bronchoalveolar lavage fluid, to a greater extent than that induced by the S morphotype (Caverly et al., 2015). Given the inflammatory nature of this immune cell subset, excessive neutrophilia can be extremely damaging to lung tissue and is a primary cause of tissue damage in CF (Downey et al., 2009). How exactly this subset contributes to protection or pathology following chronic infection is an area of continued research.

A cornerstone of the immune response to mycobacterial infection is the formation of the granuloma, a cluster of recruited host immune cell subsets which form a physical structure to restrict bacteria (Figure 2A). In zebrafish, M. abscessus granulomas are primarily comprised of neutrophils and macrophages. The accumulation of these subsets is dependent on TNF, which is required for development of the granuloma structure and the containment of bacterial growth (Bernut et al., 2016a). However, granuloma formation is not always sufficient in containing M. abscessus infection— the induction of macrophage cell death by M. abscessus releases extracellular bacteria, which form large serpentine cords that evade phagocytosis and resist immune defenses (Figure 2B; Bernut et al., 2014a; Halloum et al., 2016). Currently, the specific immunological factors differentiating protective granuloma formation from unrestrained bacterial growth are not known (Johansen et al., 2020). Murine models of M. abscessus infection also display granuloma formation in the lungs and spleen after aerosol or intravenous infection, respectively, and studies using TNF knockout (Tnf−/−) and IFN-γ knockout (Ifngr1−/− and GKO−/−) mice highlight the importance of these cytokines for maintenance of granuloma structure (Rottman et al., 2007; Ordway et al., 2008). Nude, severe combined immunodeficiency (SCID) and GMCSF knockout (GMCSF−/−) mice also develop progressive granulomatous lesions, making these useful models for preclinical drug testing but less suitable for understanding immune factors involved in granuloma formation (Obregón-Henao et al., 2015). While evidence from animal models points to a significant role of this structure in control of M. abscessus infection, the importance of granuloma formation in humans is less clear. Granuloma formation is well documented following cutaneous M. abscessus infection (Bartralot et al., 2000; Kwon et al., 2009), but fewer studies...
| **Name of virulence factor** | **Corresponding gene in reference strain** | **Distribution and biological function** | **Role of virulence factor in M. abscessus** | **Immunogenicity and/or protective efficacy** | **References** |
|----------------------------|------------------------------------------|-----------------------------------------|-------------------------------------------|--------------------------------------------|----------------|
| MgtC                       | MAB_3953                                 | Membrane bound ATPase, found in a range of CF and non-CF pathogens including *Salmonella enterica*, *Burkholderia cenocepacia* and *M. tb* | Required for optimal growth in magnesium poor media; upregulated upon intracellular macrophage infection | Ma-MgtC specific antibodies present within the serum of *M. abscessus* positive CF patients. DNA vaccination with MgtC plasmid reduces bacterial burden in Δ508 CFTR mice | Belon and Blanc-Potard, 2016; Le Moigne et al., 2016 |
| PccC                       | MAB_0555                                 | Phospholipase involved in virulence of numerous bacterial pathogens including *P. aeruginosa*, *L. monocytogenes* | Induces eukaryotic cell lysis and promotes *M. abscessus* intracellular survival in *A. castellanii* amoeba | | Le Moigne et al., 2015; N’Goma et al., 2015 |
| eccB4 (component of ESX-4) | MAB_3759c                                | Membrane component of the ESX-4 locus | Promotes *M. abscessus* survival within amoeba and macrophages by inhibition of phosphoglycogen phosphorylation and promoting phosphoglycogen-lysosomal contact | | |
| ESX-3                      | MAB_2224c-MAB_2234c                      | ESX protein secretion system | Improves *M. abscessus* survival in the animal model of infection | ΔESX-3 causes reduced inflammatory cytokine production by macrophages, reduced cell infiltration to the lungs and production of COX2 and iNOS and impaired NFκB activation in macrophages | Kim et al., 2017 |
| MAB_4780                   | MAB_4780                                 | Dehydratase, possibly involved in mycolic acid metabolism | Provides resistance to anti tubercular drug thiacetzone. Essential for extracellular cording of rough *M. abscessus*; involved in intracellular survival and granuloma formation within zebrafish model of infection | Not determined | Halloum et al., 2016 |
| Lsr2                       | MAB_0545                                 | Nucleoid associated protein/ transcriptional regulator that binds AT-rich genomic regions. Present in other mycobacterial species and essential for growth of *M. tb* | Expressed at higher levels in rough variants; Absence of Lsr2 in R variants increases susceptibility to reactive oxides and reduces intracellular survival in *A. castellanii* and macrophages. Enhances virulence in zebrafish and bacterial persistence in mice | Not determined | Le Moigne et al., 2019 |
| MmpL4b                     | MAB_4115c                                | Membrane bound protein involved in facilitating glycolipid transport to *M. abscessus* surface | Disruption of mmpL4b results in S to R transitioning, enhanced extracellular cord and abscess formation in the zebrafish model of protection. ΔmmpL4b *M. abscessus* also displays enhanced replication in macrophages | ΔmmpL4b S mutants induce TLR stimulation and production of TNF | Medjiahed and Reyrat, 2009; Nessar et al., 2011; Bernut et al., 2016b |
| MmpL8, MAB_0855            | MAB_0855                                 | Large membrane permease involved in transport of lipopolysaccharides through the plasma membrane | Promotes intracellular survival and adherence to macrophages in S colony morphotype; mutants retain ability to cause phosphoglycolipid formation but reduced ability to establish cytosolic contact | Induces IL-1β production by facilitating phagosomal-lysosomal contact | Dubois et al., 2018 |
| Pmt                        | MAB_1122c                                | Protein-O-mannosyltransferase responsible for glycosylation of lipopolysaccharides in the mycobacterial cell envelope Fatty acid | Promotes intracellular survival and adherence to macrophages in S colony morphotype; mutants retain ability to cause phosphoglycolipid formation but reduced ability to establish cytosolic contact | | Becker et al., 2017 |
|Fmt                         | Not determined                            | Fatty acid O-methyltransferase responsible for methylation of fatty acyl chain of GPL | | Δfmt displays reduced cell surface hydrophobicity and enhanced adherence to invasion of THP-1 macrophages, but no change in intracellular survival | Daher et al., 2020 |

(Continued)
characterize the granulomatous response following pulmonary infection (Jeong et al., 2004; Okazaki et al., 2013). Mycobacterium abscessus results in heterogeneous changes to pulmonary tissue following infection, including inflammatory infiltrate which may be accompanied by the formation of necrotizing or non-necrotizing granulomas (Jeong et al., 2004). Based on what is known about other NTM infections, host immune responses, clinical isolate heterogeneity, and pathogen virulence may all contribute to the extent of granuloma formation and its ability to contain M. abscessus infection (Lammas et al., 2002).

Most relevant for vaccine design is the contribution of adaptive immune subsets to M. abscessus pulmonary infection. CD4+ T helper (T\text{H}1) cells appear to be a key component of the protective response, with T\text{H}1 responses enabling control of mycobacterial dissemination and granuloma formation through...
their stimulation of innate immune subsets, such as macrophages and neutrophils (Rottman et al., 2007; Bernut et al., 2016a). In a murine model of *M. abscessus* infection, bacterial clearance is preceded by an influx of IFN-γ producing CD4+ T cells, and it has been shown that *M. abscessus* infection persists in IFN-γ knockout GKO−/− mice (Ordway et al., 2008; Obregón-Henao et al., 2015). High-dose infection models in GKO−/− mice display a heightened Th2 response resulting in an immunosuppressive phenotype, supporting the notion that Th1 polarization is ideal for protection against intracellular mycobacteria (Ordway et al., 2008). Similarly, individuals with deficiencies affecting T-cell function appear predisposed to *M. abscessus* infection (Lutzky et al., 2018). Patients with active *M. abscessus* infection also display a dampened Th1/Th2 and heightened Th17 cytokine profile compared to healthy controls, suggestive of a link between Th1 polarization and a protective phenotype (Kim et al., 2014b; Lake et al., 2016). This is an especially important consideration in the design of subunit *M. abscessus* vaccines, where adjuvant choice affects T-cell polarization and thus resulting protection (Knudsen et al., 2016).

While robust T-cell responses are clearly essential for protection against *M. abscessus*, the contribution of the humoral response to protection against pulmonary *M. abscessus* infection is less established. Antibodies to *M. abscessus* are generated following infection or vaccination (Jeon et al., 2009; Le Moigne et al., 2015) and B-cell deficiency in the murine model of infection promotes bacterial growth (Rottman et al., 2007). While humoral responses in healthy individuals are considered a critical components for extracellular pathogens, such as *P. aeruginosa*, their efficacy appears limited in preventing bacterial colonization or eradicating infection in CF patients (Yonker et al., 2015; Sainz-Mejías et al., 2020). Humoral responses that target surface GPL of *M. abscessus* S morphotypes may also encourage the conversion to the more virulent R morphotype, thus proving detrimental to the host (Gutiérrez et al., 2018). It is also unclear whether the extracellular stages of *M. abscessus* growth, such as biofilm formation during

![Figure 2](image-url)
early infection, and extracellular cording following phagosome rupture would be effectively disrupted by neutralizing antibodies. Most vaccines currently approved for human use focus on the effective development of humoral responses, and development of vaccines targeting cell-mediated immunity appear more difficult to produce. As such, the contribution of this subset to \textit{M. abscessus} protection will require continued investigation to inform development of an effective \textit{M. abscessus} vaccine.

The Quest for a \textit{Mycobacterium abscessus} Vaccine in Susceptible Populations

\textit{Mycobacterium abscessus} infection primarily occurs in individuals with reduced pulmonary immune responses, including those with CF, COPD, and non-CF bronchiectasis (Ratnatunga et al., 2020). The development of novel immunization strategies for this pathogen has the potential to dramatically reduce the \textit{M. abscessus} burden and improve quality of life for these susceptible populations. However, progress toward novel vaccines has been limited by an incomplete understanding of immune responses in these groups that perpetuate \textit{M. abscessus} infection, and how this may impact the outcome of vaccination. While populations with COPD and non-CF bronchiectasis make up a significant proportion of \textit{M. abscessus} cases, factors resulting in enhanced susceptibility of these populations to NTM infection have not yet been established (Griffith et al., 2007; Ratnatunga et al., 2020). It is speculated that structural damage within the lung in non-CF bronchiectasis permits bacterial colonization, but immune dysfunction, such as aberrant neutrophil migration and effector function, also perpetuates infection by preventing optimal clearance (Chalmers and Hill, 2013). Populations susceptible to NTM infection with no clear risk factors display reduced IFN \(\gamma\) in serum and altered adipokine levels, further suggesting an underlying link between immune responses and risk of infection (Kartalija et al., 2013).

Given the frequency with which CF populations become infected with NTM, the consideration of immune responses in CF populations is of utmost importance when developing a vaccine for \textit{M. abscessus}. As research into the area continues, we are beginning to appreciate the profound impact of CFTR mutations within CF patients on immune cell functionality, and the implications of this for pulmonary bacterial clearance (Hardt et al., 2012). The immune landscape of CF patients includes macrophages with a skewed hyperinflammatory profile (Bruscia and Bonfield, 2016), neutrophils with dysfunctional phagosomal maturation and effector functions (Zhou et al., 2013; Gifford and Chalmers, 2014), and an inflammatory milieu that may limit the ability of innate cells to clear infection (Roghanian et al., 2006; Cockx et al., 2018). Recent work by Bernut et al. also showed impaired ROS production in professional phagocytic cells following \textit{M. abscessus} infection, using a zebrafish model of CF (Bernut et al., 2019). While less is known about the implications of CFTR mutations on adaptive immune responses, lymphocytes from murine models of CF also display a predilection to the development of Thp2 and Thp17 responses (Mueller et al., 2011; Tiringer et al., 2013; Mulcahy et al., 2015). \textit{Cftr} \(^{-/+}\) mice also display dysregulated B-cell follicle formation, activation, and accumulation in the lung (Polverino et al., 2019). This adds to the complexity of vaccine development for \textit{M. abscessus}, as immune responses may be harder to predict in target populations and could potentially be naturally skewed toward non-protective phenotypes (Kartalija et al., 2013). The quality of immune responses within these populations will play a significant role in shaping the outcome of vaccination with an \textit{M. abscessus} vaccine and is therefore a key area of continued research.

Research of potential therapeutic agents to protect against \textit{M. abscessus} infection may greatly benefit from effective animal models that recapitulate the clinical presentation of \textit{M. abscessus} lung disease. This has been a continual challenge for researchers, as animal genotypes that possess common CF mutations do not display the same pathological symptoms as humans (Guilbault et al., 2007). For example, the \(\Delta F508\) mouse model possesses the most common mutation in the CFTR gene in humans but does not recapitulate the disease pathophysiology (Scholte et al., 2004). Although some knockout strains show greater persistence of bacterial load compared to wild-type littermates, none of these models develop spontaneous colonization or persistent infection by CF pathogens in the lung, such as in human patients (McMorran et al., 2001; Guilbault et al., 2005, 2007; Ordway et al., 2008; Bernut et al., 2014b). This problem has been circumvented by the use of immunocompromised strains (Lerat et al., 2014), corticosteroid administration (Maggioncalda et al., 2020), or infection with thrombin and fibrinogen plugs; a strategy similar to the agarose bead infection model used for \textit{P. aeruginosa} (Facchini et al., 2014; Caverly et al., 2015). In addition to zebrafish and \textit{A. castellanii} models, these have provided us with snapshots of the immune response to \textit{M. abscessus} infection; however, an in-depth understanding of correlates of protection is best achieved with immune competent models which recapitulate the immune response to \textit{M. abscessus} infection in humans.

The discovery of VF acquisition by \textit{M. abscessus} from other CF pathogens highlights the continued evolutionary potential of this pathogen and reinforces the urgent need for vaccines to provide robust protection to susceptible groups. Suggestions that the CF lung provides an environment that may enhance \textit{M. abscessus} virulence is particularly worrisome; however, shared VFs across different bacterial species could allow the development of protein vaccine candidates that target multiple CF pathogens (Ripoll et al., 2009; Bryant et al., 2021). For instance, PlcC antibodies from CF patients infected with \textit{P. aeruginosa} are cross-reactive with \textit{M. abscessus} PlcC (Le Moigne et al., 2015). Cross-protective vaccines are clearly advantageous in terms of research and development costs; however, no \textit{M. abscessus} vaccine study to date has confirmed a protective effect of cross-reactive immune responses induced by vaccination (Le Moigne et al., 2016). Given the number of VFs acquired from non-mycobacterial species in the \textit{M. abscessus} genome, this is a noteworthy area of continued research (Ripoll et al., 2009).

In addition to the development of vaccines for CF pathogens, there is interest in development of an \textit{M. abscessus} vaccine to provide protection against different mycobacterial species.
Lower incidence rates of *Mycobacterium avium* infection in HIV-positive individuals with prior *M. tb* infection suggest a degree of protection afforded by mycobacterial exposure (Horsburgh et al., 1996). This has encouraged interest in repurposing the current *M. tb* vaccine, *Mycobacterium bovis* Bacille Calmette–Guérin (BCG), to be used for protection against NTM infection in susceptible populations (World Health Organization, 2020). While lower incidences of NTM infection in BCG-vaccinated children support the notion of BCG vaccine-induced cross-protection, there is a paucity of information in the literature specifically referring to protection against *M. abscessus* (Trnka et al., 1994; Zimmermann et al., 2018). T cells isolated from peripheral blood of latent-TB-infected or BCG-vaccinated individuals produce IFN-γ and granzyme A in response to *M. avium* restimulation, and these cross-reactive T cells restrict growth of *M. avium* or *M. abscessus* in monocytes. BCG vaccination of mice also generates a population of T cells that secrete cytokines upon NTM restimulation; however, the effect of vaccination on bacterial burden was not determined in this study (Abate et al., 2019). As there are clear advantages of repurposing a currently approved and widely distributed vaccine, the role of BCG vaccination in providing protection against *M. abscessus* infection requires further investigation.

### Lessons Learned From TB Vaccination Efforts

Despite being heavily researched since its discovery, *M. tb* is still a leading cause of death by infectious disease worldwide. In 2020, a quarter of the world’s population was estimated to be infected with *M. tb* with 10 million new infections in 2019 alone (Chakaya et al., 2021). The protection afforded by the current vaccine available, BCG, is extremely variable across different age groups, latitudes, and to those with previous infection or mycobacterial exposure (Mangtani et al., 2014; Ahmed et al., 2021). While numerous candidates for novel TB vaccines are currently in the developmental pipeline, none have been able to provide sufficient protection to replace BCG. However, we can learn from decades of TB research and vaccine development about factors to consider in the pursuit of an *M. abscessus* vaccine, in addition to identifying unique challenges specific to this pathogen.

A robust understanding of immune responses required for protection is essential in the development of novel *M. abscessus* vaccines. In the case of vaccines against *M. tb*, efforts have been consistently stalled by our incomplete knowledge of immune correlates of protection against *M. tb* (Counoupas et al., 2019). This was exemplified in the results of the Modified Vaccinia Ankara 85A (MVA85A) vaccine in Phase IIb clinical trials, which had been a leading candidate to replace BCG. While earlier studies had shown strong T-cell-mediated immune responses following vaccination, this vaccine did not show protective efficacy in infants (Scriba et al., 2010; Tameris et al., 2013). *T. gondii* helper subsets and cytokines IFN-γ and TNF also appear to be important for protection against *M. abscessus*, but whether these subsets are predictive of protection afforded by vaccination has not yet been established (Rottman et al., 2007; Bernut et al., 2016a). The difficulty in determining immune correlates of protection against *M. abscessus* is exacerbated by the diverse growth stages of this microorganism, with extracellular biofilm formation and colonization preceding the emergence of invasive intracellular variants which form chronic infection in the lung (Howard et al., 2006; Gutiérrez et al., 2018; Bryant et al., 2021). There is currently a paucity of information on how different branches of the immune response (namely, humoral and cell-mediated subsets) contribute to effective bacterial clearance across different infection stages. Diverse vaccination strategies can be aimed at preventing colonization by inducing a strong humoral response or at enhancing clearance of persistent infection by inducing a strong cell-mediated immune response. As with *M. tb* infection, it is also unclear what impact natural immunity from prior *M. abscessus* infection has on the risk of reinfection (Verger et al., 2005; McIvor et al., 2017). Recurrence of *M. abscessus* infection has been attributed to poor antimicrobial efficacy, particularly the inefficacy of macrolide treatments to fully eradicate infection (Pasipanodya et al., 2017). However, it has been noted that reinfection with NTM, such as *M. abscessus*, frequently occurs with strains of different genotype, suggesting that *M. abscessus* antigenic variability has an impact on protective immune responses (Koh et al., 2017). A comprehensive understanding of the importance of natural immunity over the duration of *M. abscessus* infection will be critical to dictate the trajectory of novel vaccine development.

### Potential Vaccination Strategies for *Mycobacterium abscessus*

There is an enormous array of strategies that may be used in the development of a novel *M. abscessus* vaccine, each with different advantages and drawbacks. These include whole-cell (live attenuated or heat-killed), nucleic acid vaccines, and subunit (viral vectored or protein and adjuvant) vaccines.

Live attenuated vaccines have an enormous advantage in that they possess an extremely diverse repertoire of proteins that may be recognized by the immune system, thereby inducing a more diverse immune response. Beyond attenuation, whole-cell vaccines may also be modified to further enhance antigenic visibility to the immune system. VPM1002, for example, is a modified form of the BCG vaccine containing protein Listeriolysin O, which allows BCG escape into the cytosol (Grode et al., 2013). This facilitates antigen presentation to CD8+ T cells, thereby activating an additional component of the immune response to the vaccine (Grode et al., 2005). However, live attenuated vaccines pose an inherent risk of vaccine-associated disease for immunocompromised individuals or those on immunosuppressants, such as individuals with CF (Burroughs and Moscona, 2000; Malfroot et al., 2005). Heat-killed whole-cell vaccines are advantageous for these populations because there is no risk of vaccine dissemination and have been particularly successful in the TB field. One such example is Vaccae™, a whole-cell vaccine of inactivated environmental mycobacteria *Mycobacterium vaccae* (Fatima et al., 2020). Given the shared epitopes of *M. vaccae* with NTM and *M. tb*, this is a potential vaccine candidate that could be repurposed for *M. abscessus*. 

Lessons Learned From TB Vaccination Efforts

Potential Vaccination Strategies for *Mycobacterium abscessus*

---

**References**

- Abate, S., et al. (2019). The protection afforded by the current vaccine available, BCG, is extremely variable across different age groups, latitudes, and to those with previous infection or mycobacterial exposure.
- Ahmed, Z., et al. (2021). The protection afforded by the current vaccine available, BCG, is extremely variable across different age groups, latitudes, and to those with previous infection or mycobacterial exposure.
- Chakaya, J. M., et al. (2021). The protection afforded by the current vaccine available, BCG, is extremely variable across different age groups, latitudes, and to those with previous infection or mycobacterial exposure.
- Counoupas, C., et al. (2019). The protection afforded by the current vaccine available, BCG, is extremely variable across different age groups, latitudes, and to those with previous infection or mycobacterial exposure.
- Fatima, F., et al. (2020). Given the shared epitopes of *M. vaccae* with NTM and *M. tb*, this is a potential vaccine candidate that could be repurposed for *M. abscessus*.
Novel vaccine candidates with the potential of incorporating VFs also include nucleic acid-based vaccines, such as DNA vaccines. These comprise plasmids encoding antigen/s of interest and employ host cells to synthesize proteins which are recognized by the immune system. Nucleic acid vaccines are advantageous due to the relative ease of design, production and scalability. However, no DNA vaccines have progressed beyond preclinical study within the *M. tb* field—this may be because of their poorly immunogenic nature, or the more complex requirements for vaccine delivery to enter the nucleus and prevent plasmid degradation (Lee et al., 2018; Sefidi-Heris et al., 2020). Recently however, the use of tetrafunctional block copolymers to enhance cell uptake of exogenous nucleic acid has improved our ability to effectively deliver DNA vaccines (Richard-Fiardo et al., 2015). Both preclinical vaccines for *M. abscessus* are DNA formulated with tetrafunctional block polymers, one targeting *M. abscessus* VF PlcC and another targeting MgtC (McIlroy et al., 2009; Le Moigne et al., 2015, 2016). DNA vaccination against *M. abscessus* PlcC appears to provide marginally superior protection to protein-based vaccination, although the immunological mechanisms driving this trend are unclear (Le Moigne et al., 2015). However, both PlcC and MgtC DNA vaccination have a limited ability to consistently reduce bacterial burden by an appreciable level in both ΔF508 and WT mice across the course of *M. abscessus* infection (Le Moigne et al., 2015, 2016). Recent successes in mRNA vaccination for the Coronavirus Disease 2019 (COVID-19) has also promoted interest in mRNA as a vaccination strategy. This is due to the ease of manufacturing ability and the ability to induce activation of immune cells by multiple cellular pathways in response to mRNA (Brisse et al., 2020). As this area of vaccine design is relatively new, it is likely that continued research will be required to improve the potential of this formulation before use against *M. abscessus*.

Subunit vaccines comprise antigenic proteins from the target species, delivered either through viral vector or with an adjuvant to promote induction of an immune response. The selection of appropriate antigen is a critical consideration for subunit vaccines and may determine the protective outcome. Highly expressed VFs are ideal vaccine candidates, and have been targets for multiple TB vaccines in development (Chakaya et al., 2021). Importantly, antigenic expression for *M. tb* varies over the course of chronic infection and subunit vaccines targeting antigens only expressed at specific stages of infection may lack efficacy (Shi et al., 2004; Rogerson et al., 2006). CF pathogens, such as *P. aeruginosa*, also show marked change in physiology following adaptation to the CF lung, such as development of a mucoid phenotype, reduced VF expression, and loss of motility, suggesting this is a possibility for *M. abscessus* (Winstanley et al., 2016). This obstacle may be overcome through the incorporation of “early” and “late” stage antigens to target a pathogen’s full antigenic repertoire. One such example is CysVac2, a subunit vaccine which combines the secreted mycolyltransferase Ag85B with CysD, a component of the sulfate assimilation pathway expressed highly during chronic infection (Pinto et al., 2013; Counoupas et al., 2016). This highlights the importance of understanding *M. abscessus* antigen expression during infection, which may inform vaccine design.

A particular advantage of subunit vaccines is that the quality and type of the immune response may be more easily modulated through the selection of an adjuvant. Adjuvants, such as these, are most effectively used when their selection is tailored to the variety of immune response generated, which is often independent of the co-administered adjuvant (Knudsen et al., 2016). A diverse range of adjuvants are currently used in clinical TB vaccine candidates, including TLR agonists and liposomal formulations, such as IC31 and GLA-SE (Stewart et al., 2019; Enriquez et al., 2021). Most of the adjuvants being used in clinical trials for TB encourage the generation of protective Th1 and/or Th17 immune responses (Stewart et al., 2019). Given this is thought to be an immune correlate of protection against *M. abscessus*, these adjuvants may be useful components of an *M. abscessus* vaccine. Appropriate selection of adjuvant (such as one with a minimal inflammatory profile) may also facilitate non-parenteral vaccine administration, such as vaccine delivery directly to the respiratory mucosa (Ferrell et al., 2021). There is renewed interest in pulmonary vaccination for TB, whereby delivery of the vaccine directly to the lung induces tissue-resident populations capable of rapid response to *M. tb* (Counoupas et al., 2020). Similarly, intranasal vaccination with BCG provides superior protection against *M. tb* infection to subcutaneous vaccination (Perdomo et al., 2016). As our knowledge of immune responses correlated with *M. abscessus* protection increases, this may be a valuable avenue of investigation.

**Reverse Vaccinology for the Development of an Mycobacterium abscessus Vaccine**

As the field of vaccinology has advanced, so too has the use of bioinformatics approaches to aid vaccination efforts. Increases in genomic sequencing of pathogenic species has given rise to the field of “reverse vaccinology” whereby potential VFs and antigenic targets are discovered through *in silico* analysis of a pathogen’s genome and predicted proteome (Rappuoli, 2000). This method has been relatively underutilized for *M. tb*, likely due to the wealth of *in vitro* knowledge that has emerged from decades of research. A pathogen, such as *M. abscessus*, is an excellent candidate for reverse vaccinology approaches to streamline the identification of potential vaccine candidates, given the continual and growing need for a vaccine against this pathogen. There are numerous studies using panome analysis to identify shared VFs across clinical isolates of *M. abscessus*, and this is an ideal starting point for a reverse vaccinology approach (Choo et al., 2014; Davidson et al., 2014; Bryant et al., 2021; Lewin et al., 2021). There is also an extensive range of protein pathway mapping, MHC-II binding prediction, and epitope mapping software currently available which provides greater insight into those proteins likely to yield immunogenic vaccine candidates (Heinson et al., 2015). The reverse vaccinology approach has most recently been used to identify potential VFs and therapeutic targets of *M. abscessus* by development of a novel hierarchical approach; the development of such workflows is beneficial because they may be applied to other pathogens in future (Dar et al., 2021). There is also enormous advantage in a synergetic approach using wet-lab based and *in silico* techniques as a starting point for vaccine candidate identification, as this is...
based upon the confirmed proteome or secretome of different species. This was recently used by Steindor et al. to identify immunogenic proteins across different clinical isolates of *M. abscessus* and yielded many potential VFs that could be suitable vaccine targets (Steindor et al., 2019). However, current studies on *M. abscessus* reverse vaccinology lack experimental verification of protein immunogenicity; this will be a critical area of future research to determine the best candidates for progression. As we continue to build on our knowledge of this emerging pathogen, reverse vaccinology will become an essential tool in antigenic discovery for the development of subunit vaccines.

**CONCLUSION**

In recent years, we have elevated our understanding of *M. abscessus* from a relatively innocuous environmental organism to a formidable evolving true pathogen with a range of immune modulatory mechanisms to facilitate its survival within the host. These include VFs with homology to well-known mycobacterial VFs in pathogenic species, such as *M. tb*, in addition to non-mycobacterial VFs which bear striking resemblance to those present within other CF species, such as *B. cenocepacea* and *P. aeruginosa*. These VFs have a range of immune modulatory mechanisms to enhance *M. abscessus* survival within the host: promoting phagosomal escape, restriction of phagosomal acidification, enhancement of bacterial cording, and immune masking to escape detection. These, in conjunction with the extensive antibiotic resistance of this pathogen, have likely contributed to the increasing global incidence and severity of pulmonary *M. abscessus*. However, through increased understanding of *M. abscessus* virulence, we have uncovered potential vaccine candidates and novel drug targets, bringing us closer to novel prevention and eradication strategies for this pathogen. Development of a vaccine may also be guided effectively by lessons learned from decades of research into other pathogenic Mycobacteria, such as *M. tb*. Continued research into the virulence and immune correlates of protection in *M. abscessus* pathogenesis using biologically relevant animal models will provide insight into the best strategies to adopt for the development of an *M. abscessus* vaccine, to improve the quality of life of susceptible populations.

**REFERENCES**

Abate, G., Hamzabegovic, F., Eickhoff, C. S., and Hoff, D. E. (2019). BCG vaccination induces *M. avium* and *M. abscessus* cross-protective immunity. *Front. Immunol.* 10:234. doi: 10.3389/fimmu.2019.00234

Achouak, W., Heulin, T., and Pagès, J.-M. (2001). Multiple facets of bacterial virulence, in *virulence*, vol. 15, 817–826. doi: 10.1515/9780471400671

Adekambi, T., Sassi, M., van Ingen, J., and Drancourt, M. (2017). Reinstating *Mycobacterium massiliense* and *mycobacterium bolletii* subspecies Armstron, D. T., and Parrish, N. (2021). Current updates on mycobacterial pathogenesis using biologically relevant animal models will provide insight into the best strategies to adopt for the development of an *M. abscessus* vaccine, to improve the quality of life of susceptible populations.

**AUTHOR CONTRIBUTIONS**

KF and CC conceptualized the idea of the review article. KF wrote the first draft of the manuscript. KF, MJ, JT, and CC provided revision to the scientific content of the final manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

JT is supported by the NHMRC Centre of Research Excellence in Tuberculosis Control (1153493).

Funding information is available in the online version of the paper.

**ACKNOWLEDGMENTS**

JT is supported by the NHMRC Centre of Research Excellence in Tuberculosis Control (1153493).

**Supplemental Information**

The online version of this article contains supplemental material.
Belon, C., and Blanc-Potard, A.-B. (2016). Intramacrophage survival for extracellular bacterial pathogens: MgtC as a key adaptive factor. Front. Cell. Infect. Microbiol. 6:52. doi: 10.3389/fcimb.2016.00552
Belon, C., Socia, C., Bernat, A., Lauber, A., Bleves, S., and Blanc-Potard, A.-B. (2015). A macrophage death factor is shared by intracellular and extracellular pathogens. PLoS Pathog. 11:e1004969. doi: 10.1371/journal.ppat.1004969
Bentur, L., Guz, M., Ashkenazi, M., Livnat-Levanon, G., Mizrahi, M., Tal, A., et al. (2020). Pilot study to test inhaled nitric oxide in cystic fibrosis patients with refractory mycobacterium abscessus lung infection. J. Cyst. Fibros. 19, 225–231. doi: 10.1016/j.jcf.2019.05.002
Bernat, A., Dupont, C., Sahuquet, A., Herrmann, J.-L., Lutfalla, G., and Kremer, L. (2015). Deciphering and imaging pathogenesis and cording of mycobacterium abscessus in zebrafish embryos. J. Vis. Exp. 10:53130. doi: 10.3791/53130
Bernat, A., Herrmann, J.-L., Kissa, K., Dubremetz, J.-F., Gaillard, J.-L., Lutfalla, G., et al. (2014a). Mycobacterium abscessus cording prevents phagocytosis and promotes abscess formation. Proc. Natl. Acad. Sci. 111, E943–E952. doi: 10.1073/pnas.1321390111
Bernat, A., Herrmann, J.-L., Ordway, D., and Kremer, L. (2017). The diverse cellular and animal models to decipher the physiopathological traits of mycobacterium abscessus infection. Front. Cell. Infect. Microbiol. 7:100. doi: 10.3389/fcimb.2017.00100
Bernat, A., Le Moigne, V., Lesne, T., Lutfalla, G., Herrmann, J.-L., and Kremer, L. (2014b). In vivo assessment of drug efficacy against mycobacterium abscessus using the embryonic zebrafish test system. Antimicrob. Agents Chemother. 58, 4054–4063. doi: 10.1128/AAC.00142-14
Bernat, A., Nguyen-Chi, M., Halloum, I., Herrmann, J.-L., Lutfalla, G., and Kremer, L. (2016a). Mycobacterium abscessus-induced granuloma formation is strictly dependent on TNF signaling and neutrophil trafficking. PLoS Pathog. 12:e1005986. doi: 10.1371/journal.ppat.1005986
Bernat, A., Viljoen, A., Dupont, C., Sapriel, G., Blaise, M., Bouchier, C., et al. (2016b). Insights into the smooth-to-rough transition in mycobacterium bolletti unravels a functional Tyr residue conserved in all mycobacterial MmpL family members. Mol. Microbiol. 99, 866–883. doi: 10.1111/mmi.13283
Brise, M., Vrba, S. M., Kirk, N., Liang, Y., and Ly, H. (2020). Emerging concepts and technologies in vaccine development. Front. Immunol. 11:583077. doi: 10.3389/fimmu.2020.583077
Brown-Eliott, B. A., and Wallace, R. J. (2002). Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin. Microbiol. Rev. 15, 716–746. doi: 10.1128/CMR.15.4.716-746.2002
Brunson, D. N., Maldonado, E., Velez, A., Figgins, E., and Ellis, T. N. (2013). Whole-genome sequencing to identify mycobacterium abscessus. Science 339, 1828.e4–1840.e4. doi: 10.1126/science.1256675
Crawford, M. R., and Tobin, D. M. (2006). Fit for consumption: zebrafish in zebrafish embryos. J. Vis. Exp. 20:2635. doi: 10.1379/jcb.2006.06.004
Chen, J. M., German, G. J., Alexander, D. C., Ren, H., Tan, T., and Liu, J. (2010). Roles of Lsr2 in colony morphology and biofilm formation of mycobacterium smegmatis. J. Bacteriol. 192, 633–641. doi: 10.1128/JB.01788-10
Choo, S. W., Wei, Y. F., Ngew, Y. F., Mitchell, W., Tan, J. L., Wong, G. J., et al. (2014). Genomic reconnaissance of clinical isolates of emerging human pathogen mycobacterium abscessus reveals high evolutionary potential. Sci. Rep. 4, 1–10. doi: 10.1038/srep04061
Cifou, O., Riis, B., Pressler, T., Poulsen, H. E., and Helby, N. (2005). Occurrence of hypermutable Pseudomonas aeruginosa in cystic fibrosis patients is associated with the oxidative stress caused by chronic lung inflammation. Antimicrob. Agents Chemother. 49, 2276–2282. doi: 10.1128/AAC.49.6.2276-2282.2005
Cockx, M., Gouwy, M., Van Damme, J., and Struyf, S. (2018). Chemoattractants and cytokines in primary ciliary dyskinesia and cystic fibrosis: key players in chronic respiratory diseases. Cell. Mol. Immunol. 15, 312–323. doi: 10.1038/cmi.2017.118
Colangeli, R., Haq, A., Arcus, V. L., Summers, E., Magliozzo, R., McBride, A., et al. (2009). The multifunctional histione protein Lsr2 protects mycobacteria against reactive oxygen intermediates. Proc. Natl. Acad. Sci. 106, 4414–4418. doi: 10.1073/pnas.0810261016
Couopoulos, C., Ferrell, K. C., Ashhurst, A., Bhattacharyya, N. D., Nagalingam, G., Stewart, E. L., et al. (2020). Mucosal delivery of a multistage subunit vaccine promotes abscess formation of lung resident memory T cells and promotes interleukin-17-dependent protection against pulmonary tuberculosis. NPJ Vaccines 5, 1–12. doi: 10.1038/s41596-020-00255-7
Couopoulos, C., Pinto, R., Nagalingam, G., Hill-Cawthorne, A. G., Feng, C. G., Britton, W. J., et al. (2016). Mycobacterium tuberculosis components expressed during chronic infection of the lung contribute to long-term control of pulmonary tuberculosis in mice. NPJ Vaccines 1:16002. doi: 10.1038/npjvaccines.2016.12
Dar, H. A., Ismail, S., Waheed, Y., Ahmad, S., Jamil, Z., Aziz, H., et al. (2021). Designing a multi-epitope vaccine against mycobacteroides abscessus by pangenome-reverse vaccineology. Sci. Rep. 11, 1–18. doi: 10.1038/s41598-021-90868-2
Dar, H. A., Ismail, S., Waheed, Y., Ahmad, S., Jamil, Z., Aziz, H., et al. (2021). Designing a multi-epitope vaccine against mycobacteroides abscessus by pangenome-reverse vaccineology. Sci. Rep. 11, 1–18. doi: 10.1038/s41598-021-90868-2
Daher, W., Leclercq, L.-D., Viljoen, A., Karam, J., Dufrene, Y. F., Guérardel, Y., et al. (2020). O-methylation of the glycopeptidolipid acyl chain defines surface hydrophobicity of mycobacterium abscessus and macrophage invasion. ACS Infect. Dis. 6, 2756–2770. doi: 10.1021/acsinfecdis.0c00490
Dar, H. A., Ismail, S., Waheed, Y., Ahmad, S., Jamil, Z., Aziz, H., et al. (2021). Designing a multi-epitope vaccine against mycobacteroides abscessus by pangenome-reverse vaccineology. Sci. Rep. 11, 1–18. doi: 10.1038/s41598-021-90868-2
Ferrell, K. C., Stewart, E. L., Counoupas, C., Ashhurst, T. M., Britton, W. J., Feng, Z., Bai, X., Wang, T., Garcia, C., Bai, A., Li, L., et al. (2020). Differential responses by human macrophages to infection with mycobacterium tuberculosis and non-tuberculous mycobacteria. Front. Microbiol. 11:116. doi: 10.3389/fmicb.2020.00116

Ferrell, K. C., Stewart, E. L., Counoupas, C., Ashhurst, T. M., Britton, W. J., Petrovsky, N., et al. (2021). Intrapulmonary vaccination with delta-inulin adjuvant stimulates non-polarised chemotactic signalling and diverse cellular interaction. Mucosal Immunol. 14, 762–773. doi: 10.1038/s41385-021-00357-9

Folkesson, A., Jelkhāb, L., Yang, L., Johansen, H. K., Ciufò, O., Hulby, N., et al. (2012). Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: an evolutionary perspective. Nat. Rev. Microbiol. 10, 841–851. doi: 10.1038/nrmicro2907

Fothergill, I. L., Walsh, M. J., and Winstanley, C. (2012). Transmissible strains of Pseudomonas aeruginosa in cystic fibrosis lung infections. Eur. Respir. J. 40, 227–238. doi: 10.1183/09031936.00204411

Ganbat, D., Seehase, S., Richter, E., Vollmer, E., Reiling, N., Fellenberg, K., et al. (2016). Mycobacteria infect different cell types in the human lung and cause species dependent cellular changes in infected cells. BMC Pulm. Med. 16, 1–16. doi: 10.1186/s12890-016-0185-5

Ghannoum, M. A. (2000). Potential role of phospholipases in virulence and fungal pathogenesis. Clin. Microbiol. Rev. 13, 122–143. doi: 10.1128/cmrr.13.1.122

Gifford, A. M., and Chalmers, J. D. (2014). The role of neutrophils in cystic fibrosis. Curr. Opin. Hematol. 21, 16–22. doi: 10.1097/MOH.0000000000000009

Gordon, B. R., Li, Y., Wang, L., Sintsova, A., Van Bakel, H., Tian, S., et al. (2010). Lr2 is a nucleoid-associated protein that targets AT-rich sequences and virulence genes in mycobacterium tuberculosis. Proc. Natl. Acad. Sci. 107, 5154–5159. doi: 10.1073/pnas.0913551107

Griffith, D. F., Akinsat, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., et al. (2007). An official ATS/IDSA statement: diagnosis, treatment, and infection control of nontuberculous mycobacterial diseases. Clin. Infect. Dis. 44, e221–e226. doi: 10.1086/520174

Grode, L., Ganoza, C. A., Brohm, C., Weiner, I., Eisele, B., and Kaufmann, S. H. (2013). Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. Vaccine 31, 1340–1348. doi: 10.1016/j.vaccine.2012.12.053

Grode, L., Seiler, P., Baumann, S., Hess, J., Brinkmann, V., Eddine, A. N., et al. (2005). Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants that secrete listeriolysin. J. Clin. Invest. 115, 2472–2479. doi: 10.1172/JCI24617

Gröschel, M., I., Sayes, F. Simeone, R., Majlessi, L., and Brosch, R. (2016). ESX secretion systems: mycobacterial evolution to counter host immunity. Nat. Rev. Microbiol. 14, 677–691. doi: 10.1038/nrmicro2016.131

Guibault, C., Martin, P., Houle, D., Bogdahy, M., Guiot, M., Marion, D., et al. (2005). Cystic fibrosis lung disease following infection with Pseudomonas aeruginosa in Cfr knockout mice using novel non-invasive direct pulmonary infection technique. Lab. Anim. 39, 336–352. doi: 10.1258/0023677054306944

Guibault, C., Saeed, Z., Downey, G. P., and Radzioch, D. (2007). Cystic fibrosis mouse models. Am. J. Respir. Cell Mol. Biol. 36, 1–7. doi: 10.1165/rcmb.2006-0184TR

Guimarães, T., Chimara, E., do Prado, G. V. B., Ferrazoli, L., NGF, C., dos Santos, L. D., et al. (2012). Adaptation of mycobacterium tuberculosis to human environment. An evolutionary perspective. J. Clin. Immunol. 32, 107–121. doi: 10.1007/s10875-011-9606-z

Guimarães, T., Chimara, E., da Silva, J. L., Nguyen, J., Fennelly, K. P., Zelazny, A. M., and Olivier, K. N. (2016). Mycobacterium abscessus subsp bolletii in a digestive and respiratory endoscopy unit caused by the same clone as that of a countrywide outbreak. Clin. Infect. Dis. 63, 165–171. doi: 10.1093/cid/ciw099

Halloum, I., Carrere-Kremer, S., Blaise, M., Viljoen, A., Bernut, A., Le Moigne, V., et al. (2016). Pseudooutbreak of rapidly growing mycobacteria in a digestive and respiratory endoscopy unit caused by the same clone as that of a countrywide outbreak. Am. J. Infect. Control 44, e221–e226. doi: 10.1016/j.ajic.2016.06.019

Gupta, R. S., Lo, B., and Son, J. (2018). Phylogenomics and comparative genomic studies robustly support division of the genus mycobacterium into an emended genus mycobacterium and four novel genera. Front. Microbiol. 9:1145. doi: 10.3389/fmicb.2018.01145

Gutiérrez, A. V., Viljoen, A., Ghigo, E., Herrmann, J.-L., and Kremer, L. (2018). Glycopeptidolipids, a double-edged sword of the mycobacterium. Front. Microbiol. 9:1032. doi: 10.3389/fmicb.2018.01032

Halloum, I., Carrere-Kremer, S., Blaise, M., Viljoen, A., Bernut, A., Le Moigne, V., et al. (2016). Deletion of a dehydratase important for intracellular growth and cording renders rough mycobacterium abscessus avirulent. Proc. Natl. Acad. Sci. U. S. A. 113, E4228–E4237. doi: 10.1073/pnas.1605771113

Halloum, I., Viljoen, A., Khanna, V., Craig, D., Bouchier, C., Brosch, R., et al. (2017). Resistance to thiacyetazone derivatives active against mycobacterium abscessus involves mutations in the MmpL5 transcriptional repressor MAB_4384. Antimicrob. Agents Chemother. 61:e02509-16. doi: 10.1128/AAC.02509-16
chronic respiratory infection. Int. J. Med. Microbiol. 304, 1182–1191. doi: 10.1016/j.ijmm.2014.08.011

Martiniano, S. L., Nick, J. A., and Daley, C. L. (2019). Nontuberculous mycobacterial infections in cystic fibrosis. Thorac. Surg. Clin. 29, 95–108. doi: 10.1016/j.tscl.2018.09.008

McIlroy, D., Barteau, B., Cany, I., Richard, P., Gourdin, C., Conchon, S., et al. (2009). DNA/ampiphilic block copolymer nanospheres promote low-dose DNA vaccination. Mol. Ther. 17, 1473–1481. doi: 10.1038/mt.2009.84

McVor, A., Koornhof, H., and Kana, B. D. (2017). Relapse, re-infection and mixed infections in tuberculosis disease. Pathog. Dis. 75:3. doi: 10.1093/femsmp/ftx020

McMorran, B., Palmer, J., Lunn, D., Oceandy, D., Costelloe, E., Thomas, G., et al. (2001). G551D CF mice display an abnormal host response and have impaired clearance of pseudomonas lung disease. Am. J. Phys. Lung Cell. Mol. Phys. 281, L740–L747. doi: 10.1152/ajplung.2001.281.3.L740

Medjahed, H., and Reyrat, J.-M. (2009). Construction of mycobacterium abscessus defined glycopeptidolipid mutants: comparison of genetic tools. Appl. Environ. Microbiol. 75, 1331–1338. doi: 10.1128/AEM.01914-08

Meir, M., Grosfeld, T., and Barkan, D. (2018). Establishment and validation of galleria mellonella as a novel model organism to study mycobacterium abscessus infection, Pathogenesis, and Treatment. Antimicrob. Agents Chemother. 62, e02539–e025317. doi: 10.1128/AAC.02539-17

Melly, G., and Purdy, G. E. (2019). MmpL proteins in physiology and pathogenesis of M. tuberculosis. Microorganisms 7:70. doi: 10.3390/microorganisms7030070

Menard, E., Madh, W., Ericossous, B., Rodrguez, D., and Sadkot, R. T. (2013). Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. J. Infect. Dis. 17, e1000–e1004. doi: 10.1093/infdis/jij3.103.018

Mueller, C., Braag, S. A., Keeler, A., Hodges, C., Drumm, M., and Flotte, T. R. (2011). Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. Am. J. Respir. Cell Mol. Biol. 44, 922–929. doi: 10.1165/rcmb.2010-0224OC

Mulcahy, E. M., Hudson, J. B., Beggs, S. A., Reid, D. W., Roddam, L. F., and Cooley, M. A. (2015). High peripheral blood th17 percent associated with poor lung function in cystic fibrosis. PLoS One 10:e0129912. doi: 10.1371/journal.pone.0129912

Muller, A., Gunther, D., Dux, F., Naumann, M., Meyer, T. F., and Rudel, T. (1999). Neisserial porin (PorB) causes rapid calcium influx in target cells and induces apoptosis by the activation of cytochrome poxenes. EMBO J. 18, 339–352. doi: 10.1093/emboj/18.2.339

Nesser, R., Cambau, E., Reyrat, J. M., Murray, A., and Gicquel, B. (2012). Mycobacterium abscessus: a new antibiotic nightmare. J. Antimicrob. Chemother. 67, 810–818. doi: 10.1093/jac/dkr578

Nesser, R., Reyrat, J.-M., Davidson, L. B., and Byrd, T. F. (2011). Deletion of the mphA gene in the mycobacterium abscessus glycopeptidolipid biosynthetic pathway results in loss of surface colonization capability, but enhanced ability to replicate in human macrophages and stimulate their innate immune response. Microbiology 157, 1187–1195. doi: 10.1099/mic.0.046557-0

N’Goma, J. C. B., Le Moigne, V., Soisnier, M., Laencina, L., Le Chevalier, F., Roux, A.-L., et al. (2015). Mycobacterium abscessus phospholipase C expression is induced during coculture within amoebae and enhances M. abscessus virulence in mice. Infect. Immun. 83, 780–791. doi: 10.1128/IAI.00232-14

Obreng-Henao, A., Arnett, K. A., Henao-Tamayo, M., Massoudi, L., Creissen, E., Andries, K., et al. (2015). Susceptibility of mycobacterium abscessus to antinmycobacterial drugs in preclinical models. Antimicrob. Agents Chemother. 59, 6904–6912. doi: 10.1128/AAC.00459-15

Okaizaki, A., Takato, H., Ohkura, N., Katayama, N., Kasahara, K., and Fujimura, M. (2013). Successful treatment with chemotherapy and corticosteroids of mycobacterium abscessus infection accompanied by pleural effusion. J. Infect. Chemother. 19, 964–968. doi: 10.1007/s10156-012-0359-9

Ordway, D., Henao-Tamayo, M., Smith, E., Shanley, C., Harton, M., Troudt, J., et al. (2008). Animal model of mycobacterium abscessus lung infection. J. Leukoc. Biol. 83, 1502–1511. doi: 10.1189/jlb.0707927

Oberley-Deegan, R. E., Rebits, B. W., Weaver, M. R., Tollefson, A. K., Bai, X., McGhney, M., et al. (2010). An oxidative environment promotes growth of mycobacterium abscessus. Free Radic. Biol. Med. 49, 1666–1673. doi: 10.1016/j.freeradbiomed.2010.08.026

Ordway, D., Henao-Tamayo, M., Smith, E., Shanley, C., Barton, M., Trott, J., et al. (2008). Animal model of mycobacterium abscessus lung infection. J. Leukoc. Biol. 83, 1502–1511. doi: 10.1189/jlb.0707927
Stewart, E., Tricas, J. A., and Petrovsky, N. (2019). Adjuvant strategies for more effective tuberculosis vaccine immunity. Microorganisms 7:255. doi: 10.3390/microorganisms7080255

Sturgill-Koszyki, S., Schlesinger, P. H., Chakraborty, P., Haddix, P. L., Collins, H. L., Fok, A. K., et al. (1994). Lack of acidification in mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science 263, 678–681. doi: 10.1126/science.8303277

Tam, P.-Y. L., Kline, S., Wagner, J. E., Guspiel, A., Streifel, A., Ward, G., et al. (2014). Rapidly growing mycobacteria among pediatric hematopoietic cell transplant patients traced to the hospital water supply. Pediatr. Infect. Dis. J. 33, 1043–1046. doi: 10.1097/INF.0000000000000391

Tameris, M. D., Hatherill, M., Landry, B. S., Scriba, T. J., Snowden, M. A., Lockhart, S., et al. (2013). Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 381, 1021–1028. doi: 10.1016/S0140-6736(13)60177-4

Tan, L. T.-H., Raghunath, P., Ming, L. C., and Law, J. W.-F. (2020). Mycobacterium ulcerans and Mycobacterium marinum: pathogenesis, diagnosis and treatment. Prog. Microbes Mol. Biol. 3:1. doi: 10.36877/pmmb.a0000114

Thomas, V., and McDonnell, G. (2007). Relationship between mycobacteria and amoebaec: ecological and epidemiological concerns. Lett. Appl. Microbiol. 45, 349–357. doi: 10.1111/j.1742-765X.2007.02206.x

Thomson, R., Tolson, C., Siddhant, H., Huyngs, F., and Hargreaves, M. (2013). Mycobacterium abscessus isolated from municipal water—a potential source of human infection. BMC Infect. Dis. 13, 1–7. doi: 10.1186/1471-2334-13-241

Tiringer, K., Treis, A., Fucik, P., Gona, M., Gruber, S., Renner, S., et al. (2013). A Th17 and Th2-skewed cytokine profile in cystic fibrosis lungs represents a potential risk factor for Pseudomonas aeruginosa infection. Am. J. Respir. Crit. Care Med. 187, 621–629. doi: 10.1164/rccm.201206-1150OC

Tortoli, E., Fedrizzi, T., Meehan, C. J., Trovato, A., Grottolia, A., Giacobazzi, E., et al. (2017). The new phylogeny of the genus mycobacterium: the old and new. Infect. Genet. Evol. 56, 19–25. doi: 10.1016/j.meegid.2017.10.013

Trnka, L., Danková, D., and Svandová, E. (1994). Six years’ experience with the discontinuation of BCG vaccination: 4. Protective effect of BCG vaccination against the Mycobacterium avium intracellulare complex. Tuber. Lung Dis. 75, 348–352. doi: 10.1016/0962-8479(94)90080-9

Tsai, S. H., Shen, G. H., Lin, C. H., Liao, J. R., Lai, H. C., and Hu, S. T. (2013). Mah3168c, a putative acetyltransferase, enhances adherence, intracellular survival and antimicrobial resistance of mycobacterium abscessus. PLoS One 8:e67563. doi: 10.1371/journal.pone.0067563

Tufariello, J. M., Chapman, J. R., Kerantzas, C. A., Wong, K.-W., Vilchez, C., Jones, C. M., et al. (2016). Separable roles for mycobacterium tuberculosis ESX-3 effectors in iron acquisition and virulence. Proc. Natl. Acad. Sci. 113, E348–E357. doi: 10.1073/pnas.152321113

van der Wel, N., Hava, D., Houben, D., Fluitema, D., van Zon, M., Pierson, I., et al. (2007). M. Tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell 129, 1287–1298. doi: 10.1016/j.cell.2007.05.059

Verver, S., Warren, R. M., Beyers, N., Richardson, M., Van Der Spuy, G. D., Borgdorff, M. W., et al. (2005). Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. Am. J. Respir. Crit. Care Med. 171, 1430–1435. doi: 10.1164/rccm.200409-1200OC

Viljoen, A., Dubois, V., Girard-Misquich, F., Blaise, M., Herrmann, J. L., and Kremer, L. (2017). The diverse family of M mpL transporters in mycobacteria: from regulation to antimicrobial developments. Mol. Microbiol. 104, 889–904. doi: 10.1111/mmi.13675

Viljoen, A., Viela, F., Kremer, L., and Dufrêne, Y. F. (2020). Fast chemical force microscopy demonstrates that glycopeptidolipids define nanodomains of varying hydrophobicity on mycobacteria. Nanoscale Horiz. 5, 944–953. doi: 10.1039/C9NH00736A

Weerdemu, E. M., Abdallah, A. M., Mitra, S., De Punder, K., Van Der Wel, N. N., Bird, S., et al. (2012). ESX-5-deficient Mycobacterium marinum is hypervirulent in adult zebrafish. Cell. Microbiol. 14, 728–739. doi: 10.1111/j.1462-5822.2012.01755.x

Wiang, J., Back, Y. W., Lee, K. I., Fijiwara, N., Paik, S., Choi, C. H., et al. (2017). Mycobacterium abscessus glycopeptidolipids inhibit macrophage apoptosis and bacterial spreading by targeting mitochondrial cyclophilin D. Cell Death Dis. 8:e3012. doi: 10.1038/cddis.2017.420

Wiersma, C. J., Belardielli, J. M., Avanzí, C., Angala, S. K., Everall, I., Angala, B., et al. (2020). Cell surface remodeling of mycobacterium abscessus under cystic fibrosis airway growth conditions. ACS Infect. Dis. 6, 2143–2154. doi: 10.1021/acsinfecdis.0c00214

Winstanley, C., O’Brien, S., and Brockhurst, M. A. (2016). Pseudomonas aeruginosa evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. Trends Microbiol. 24, 327–337. doi: 10.1016/j.tim.2016.01.008

World Health Organization (2020). Global tuberculosis report 2020: executive summary. Available at: https://www.who.int/teams/global-tuberculosis-programme/th-reports/global-tuberculosis-report-2021 (Accessed December 12, 2021).

Wu, H.-J., Wang, A. H., and Jennings, M. P. (2008). Discovery of virulence factors of pathogenic bacteria. Curr. Opin. Chem. Biol. 12, 93–101. doi: 10.1016/j.cbpa.2007.10.001

Yonker, L. M., Cigan, C., Hurley, B. P., and Bragonzi, A. (2015). Host-pathogen interplay in the respiratory environment of cystic fibrosis. J. Cyst. Fibros. 14, 431–439. doi: 10.1016/j.jcf.2015.02.008

Zhang, C., Asif, H., Holt, G. E., Griswold, A. J., Campos, M., Bejarano, P., et al. (2019). Mycobacterium abscessus—bronchial epithelial cells cross-talk through type I interferon signaling. Front. Immunol. 10:2888. doi: 10.3389/fimmu.2019.02888

Zhou, Y., Song, K., Painter, R. G., Aiken, M., Reiser, J., Stanton, B. A., et al. (2013). Cystic fibrosis transmembrane conductance regulator recruitment to phagosomes in neutrophils. J. Innate Immun. 5, 219–230. doi: 10.1159/000346568

Zimmermann, P., Finn, A., and Curtis, N. (2018). Does BCG vaccination protect against nontuberculous mycobacterial infection? A systematic review and meta-analysis. J. Infect. Dis. 218, 679–687. doi: 10.1093/infdis/jiy207

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Ferrell, Johansen, Tricas and Cououpas. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.