



Mycobacterial virulence: impact on immunogenicity and vaccine research

Vera Kroesen, Jan Madacki, Wafa Frigui, Fadel Sayes, Roland Brosch

► To cite this version:

Vera Kroesen, Jan Madacki, Wafa Frigui, Fadel Sayes, Roland Brosch. Mycobacterial virulence: impact on immunogenicity and vaccine research. F1000Research, 2019, 8, pp.2025. 10.12688/f1000research.20572.1 . pasteur-02615238

HAL Id: pasteur-02615238

<https://pasteur.hal.science/pasteur-02615238>

Submitted on 22 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



REVIEW

Mycobacterial virulence: impact on immunogenicity and vaccine research [version 1; peer review: 4 approved]

Vera M. Kroesen^{1,2}, Jan Madacki¹, Wafa Frigui ¹, Fadel Sayes ¹, Roland Brosch ¹

¹Unit for Integrated Mycobacterial Pathogenomics, CNRS UMR 3525, Institut Pasteur, Paris, France

²Faculty VI, University of Oldenburg, Oldenburg, Germany

V1 First published: 28 Nov 2019, 8(F1000 Faculty Rev):2025 (<https://doi.org/10.12688/f1000research.20572.1>)

Latest published: 28 Nov 2019, 8(F1000 Faculty Rev):2025 (<https://doi.org/10.12688/f1000research.20572.1>)

Abstract

The borderline between virulence and efficacy in live attenuated vaccine strains is often blurred and this is also the case for the Bacillus Calmette–Guérin (BCG), the only currently licensed anti-tuberculosis vaccine used on a large, global scale, which was obtained almost 100 years ago. While BCG is more than 99% identical at the genome level to *Mycobacterium tuberculosis*, the causative pathogen of human tuberculosis, some important differences in virulence factors cause naturally irreversible attenuation and safety of this vaccine in the immunocompetent host. Some of these virulence factors are involved in persistence capacities of the vaccine strains and also represent strong immunogens, responsible for inducing different host signaling pathways, which have to be taken into consideration for the development of revised and new vaccine strains. Here we discuss a number of selected mycobacterial features in relation to their biological functions and potential impact on virulence and vaccine efficacy.

Keywords

tuberculosis, mycobacteria, host adaption, virulence, pathogen evolution, vaccination

Open Peer Review

Reviewer Status

	Invited Reviewers			
	1	2	3	4
version 1				
published 28 Nov 2019				

F1000 Faculty Reviews are written by members of the prestigious F1000 Faculty. They are commissioned and are peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

- 1 **Helen Fletcher** , London School of Hygiene and Tropical Medicine, London, UK
- 2 **Ramandeep Singh**, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, India
- 3 **Jun Liu**, University of Toronto, Toronto, Canada
- 4 **Manuel Elkin Patarroyo**, Universidad Nacional de Colombia, Carrera, Colombia

Any comments on the article can be found at the end of the article.

Corresponding author: Roland Brosch (roland.brosch@pasteur.fr)

Author roles: **Kroesen VM:** Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Madacki J:** Conceptualization, Writing – Review & Editing; **Frigui W:** Investigation, Writing – Review & Editing; **Sayes F:** Conceptualization, Supervision, Writing – Review & Editing; **Brosch R:** Conceptualization, Funding Acquisition, Resources, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Research in Roland Brosch's laboratory is in part supported by the European Union's Horizon 2020 Research and Innovation Program (grant 643381 TBVAC2020), the Agence National de Recherche (ANR-10-LABX-62-IBEID, ANR-16-CE15-0003, and ANR-16-CE35-0009), and the Fondation pour la Recherche Médicale FRM (DEQ20130326471). The research internship of Vera M. Kroesen at the Institut Pasteur in Paris was supported by a PROMOS grant from the German Academic Exchange Service (DAAD) and the Institut Pasteur. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2019 Kroesen VM *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Kroesen VM, Madacki J, Frigui W *et al.* **Mycobacterial virulence: impact on immunogenicity and vaccine research [version 1; peer review: 4 approved]** F1000Research 2019, 8(F1000 Faculty Rev):2025 (<https://doi.org/10.12688/f1000research.20572.1>)

First published: 28 Nov 2019, 8(F1000 Faculty Rev):2025 (<https://doi.org/10.12688/f1000research.20572.1>)

Introduction

Tuberculosis (TB) remains among the top 10 causes of death worldwide¹. In 2017, an estimated 10 million people developed new TB disease, of which 90% were aged 15 or older¹. This lacking control of TB in a large number of people emphasizes the complex disease TB represents, involving a highly persistent bacterium, *Mycobacterium tuberculosis*, and the variable abilities of individual human immune systems to cope with this infection. As TB disease may also occur in certain individuals who have been vaccinated during early childhood with the widely used attenuated *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) vaccine, this situation also points to the shortcomings of BCG, the only licensed vaccine against TB today. BCG provides protection against severe, disseminated TB in children, but protection often fades in adolescence and fails to protect against pulmonary TB. The reported efficacy of BCG against adult pulmonary TB varies hugely (0–80%) and seems to depend on many factors, including social conditions, co-infection with helminths, and other variables^{2–5}. BCG can generally be regarded as safe, although individuals with immune deficiencies can develop disseminated BCG disease³. Thus, despite the millions of childhood TB cases prevented by large-scale BCG vaccination strategies, there is a need for a more effective vaccine that can interrupt the vicious infection cycle of *M. tuberculosis*, which is driven by adolescent and adult patients with pulmonary TB disease. In this review, we will thus focus on selected mycobacterial virulence factors that play a role as potent immunogens as well as potential targets for the attenuation of novel vaccine strains.

Virulence factors in *Mycobacterium tuberculosis*

An accurate definition of mycobacterial virulence is quite difficult to find, as many factors may influence the survival of *M. tuberculosis* in specific environmental conditions. Apart from essential genes that are needed by *M. tuberculosis* for its survival in commonly used mycobacterial growth media^{6,7}, its survival in the hostile environment inside host phagocytes requires additional skills from the bacterium. An overview of such *in vivo*-required genes was obtained by using libraries of transposon mutants of *M. tuberculosis* strains in murine infection models, unraveling between 200 and 500 genes essential for the growth of *M. tuberculosis in vivo*^{8,9}. Among these genes, several were also identified as important virulence determinants of *M. tuberculosis* by comparative analyses of attenuated and virulent tubercle bacilli, including genes encoding proteins involved in secretion systems, persistence mechanisms, or lipid metabolism^{10–17}, as further discussed below.

ESX/ESAT-6/type VII secretion systems

ESX secretion systems are specialized bacterial secretion systems needed for the transport of substrates through the complex, thick mycobacterial cell envelope, which is composed of an inner phospholipid bilayer and an outer membrane (also called the mycomembrane) largely composed of mycolic acids, at the inner side covalently attached to arabinogalactan, which is in turn attached to a layer of peptidoglycan embedded in the periplasm, separating the inner and outer membrane^{18,19}. Variable numbers of these secretion systems are present in

mycobacteria and other actinobacteria, such as *Streptomyces* or *Corynebacteria*, and more distantly related ESX-like systems are also found in Gram-positive bacteria, such as *Staphylococcus aureus*^{18,20}. In mycobacteria, five distinct ESX secretion systems are known to date, ESX-1 to ESX-5, of which at least three, ESX-1, ESX-3, and ESX-5, are needed for full virulence in *M. tuberculosis*. The roles of ESX-2 and ESX-4 are not yet understood¹⁸. ESAT-6 (6 kDa early secretory antigenic target/EsxA) as the first identified effector protein has led to the nomenclature of the secretion systems as ESX or ESAT-6 secretion systems, though, of the five ESX secretion systems known to date, only ESX-1 is known to specifically secrete ESAT-6¹⁸. Another commonly used name for ESX systems is type VII secretion (T7S) systems, in analogy to the well-studied secretion systems in Gram-negative bacteria²¹.

The ESX-1 secretion system within the region of difference 1 (RD1)

ESX-1, the first-described ESX secretion system, was discovered by comparative genomics approaches between virulent *M. bovis* and/or *M. tuberculosis* and attenuated mycobacterial strains, such as BCG and *Mycobacterium microti*^{10,22,23}. Indeed, both BCG and *M. microti* are attenuated members of the *M. tuberculosis* complex (MTBC) that have been widely used for human vaccination^{24,25}, and, interestingly, both vaccine strains lack overlapping portions of the genomic region of difference 1 (RD1), encoding the ESX-1 secretion system.

The ESX-1 secretion system is a protein complex localized in the mycobacterial plasma membrane. The complex is composed of the ESX conserved components EccB, EccD, and EccE and the ATP-dependent translocase EccC^{26,27}. The structural organization of the ESX-1 secretion machinery might closely resemble that of the ESX-5 system of *Mycobacterium xenopi*, for which cryoelectron-microscopy-based single particle analysis has revealed a hexameric organization²⁸. Intriguingly, a hexameric structure was also demonstrated for the ESX-3 system of *Mycobacterium smegmatis*, which consists of four protein components: EccB3, EccC3, EccD3, and EccE3 in a 1:1:2:1 stoichiometry²⁹. The main effector protein ESAT-6 is likely secreted by ESX-1 as a heterodimer together with EsxB/CFP-10 (10 kDa culture filtrate protein) in a 1:1 ratio³⁰, although under certain circumstances the two protein partners might also be secreted at other ratios^{16,27}. As another particularity of the ESX-1 machinery, the secretion of ESAT-6/CFP-10 is dependent on ESX-1-associated proteins EspC and EspA, which in turn seem dependent on the ATPase EccA located in the cytosol and EspD for stabilization^{19,31–33}. The functions of EspA and EspC are unknown, but EspC was speculated to form tunnel-like filaments, allowing the secretion of effector proteins through the outer membrane³⁴. Interestingly, the ESX-1-associated EspA, EspC, and EspD are encoded in genomic islands that seem to have been acquired by independent horizontal gene transfer events in several groups of pathogenic mycobacteria at different genomic locations³⁵. EspA and EspC do show some sequence similarity to EspE, EspF, and EspH, which are proteins that are encoded together with the putative chaperone EspG at the 5' end of the *esx-1* locus. While the deletion of EspF and EspG did not impact the secretion of ESAT-6 and

CFP-10, the lack of these proteins attenuated the mutant *M. tuberculosis* strain³⁶. In other experiments, the retained ESAT-6 secretion activity in EspF and EspG deletion mutants was confirmed by using an intraphagocytic release assay³⁷. In addition, *espE* is one of the *esx-1* genes repeatedly identified by large-scale transposon screens as being important for virulence^{8,9}. There are several other ESX-1-associated proteins encoded by the *esx-1* locus. Sala and co-workers recently reported that EspL is essential for ESX-1-mediated secretion and proposed a model in which EspL associates with EspD to act as a chaperone for EspF, the dimer EspH/EspE, and the dimer EspC/EspA. The latter are needed for the secretion of ESAT-6/CFP-10³³. EspB is another substrate of ESX-1 and has been described to form folds similar to another type of ESX effector protein, namely PE/PPE proteins, containing a similar N-terminal region to PE/PPE. It has been shown that EspB is processed by the protease MycP1 during secretion, and its role in the pathogenicity of *M. tuberculosis* is not yet understood^{19,38}.

The PE/PPE protein families and the ESX-5 secretion system

The effector proteins ESAT-6 and CFP-10 secreted by ESX-1 belong to the so-called WXG100 protein family, named after the characteristic WxG hairpin motif³⁹, which is present in the secreted, small Esx proteins of the different ESX systems⁴⁰. Other important effectors of the ESX secretion apparatus which often also carry a WxG motif belong to the large PE/PPE protein families, which are not specific to ESX-1⁴¹. PE/PPE are polymorphic proteins that share an N-terminal Pro-Glu (PE) or Pro-PE (PPE) signature and similarities in structural folding^{42,43}. The majority of PE/PPE proteins is secreted by the ESX-5 secretion system, which thus controls the export of a multiplicity of immunogens and potential virulence factors¹⁴. The ESX-5 secretion system is the most recently evolved ESX system and is harbored only by slow-growing mycobacteria^{44,45}. The *esx-5* locus includes five genes encoding secreted PE/PPE proteins, named PPE25 (Rv1787), PE18 (Rv1788), PPE26 (Rv1789), PPE27 (Rv1790), and PE19 (Rv1791), which seem to be the result of gene duplication events of a PE/PPE encoding gene pair, as shown by their high sequence similarity. In addition, many other PE/PPE proteins, which are not directly encoded within the *esx-5* locus, are also exported via the ESX-5 apparatus^{43,46}. The ESX-5-encoded PE/PPE proteins have been found to be highly immunogenic^{43,46} and to represent potential virulence factors, as shown by the attenuation of an *M. tuberculosis* deletion mutant lacking the five *esx-5*-associated *pe/ppe* genes¹⁴. In the *M. tuberculosis* H37Rv reference strain, the PPE25, PPE26, and PPE27 proteins belong to the 24-membered PPE-GxxSVPxxW subgroup of PPE proteins⁴⁷, which share the particular GxxSVPxxW sequence motif. Another prominent member of this subgroup is the PPE18 protein (also known as Rv1196 or Mtb39A), which together with PepA (also known as Rv0125 or Mtb32A) and the adjuvant AS01E constitutes the novel M72/AS01E vaccine candidate, currently in clinical evaluation^{48–51}. Moreover, another member of the PPE-GxxSVPxxW subgroup is PPE38 (Rv2352c), which was shown to be needed for the export of certain subclasses

of PE and PPE proteins in addition to the ESX-5 system¹⁷. Indeed, it was found that export of PE_PGRS proteins that are encoded by genes with polymorphic GC-rich sequences, as well as PPE proteins with major polymorphic tandem repeats (PPE-MPTR subgroup), requires the presence of ESX-5 and a functional PPE38 protein. Certain lineages of *M. tuberculosis* strains, such as most of the members of the *M. tuberculosis* lineage 2 (Beijing) strains, lack parts of the genetic region encoding PPE38 and are thus unable to secrete a large number of PE_PGRS and PPE-MPTR proteins¹⁷. As phenotypic consequences of such secretion defects, a gain of virulence in certain *M. tuberculosis* strains has been observed in murine infection models¹⁷. However, the mechanism by which the secretion of PE_PGRS and PPE_MPTR proteins may interfere with virulence remains unknown. It has also been observed that *M. bovis* and the *M. bovis*-derived BCG vaccine strains lack PPE38 due to the deletion of the RD5 region and therefore do not export the plethora of PE_PGRS and PPE_MPTR proteins⁵². While genetic introduction of a *ppe38* gene copy into the genome of BCG resulted in recombinant BCG38 showing a re-established secretion phenotype, no significant differences in vaccine efficacy between wild-type BCG and BCG38 have been found in murine infection systems⁵². However, the finding that the BCG strains currently used in human vaccination do not export certain PE and PPE proteins remains an important discovery for vaccine developers, particularly as certain booster vaccines use PPE proteins as their targets, for which it is uncertain whether they were properly exported by BCG and exposed to the immune system during primary vaccination^{48,53–55}. Thus, the elucidation of the function of PE and PPE proteins and their contribution to virulence and immunogenicity remains one main research priority in TB research.

Lipid surface factors

The outer membrane of mycobacteria is largely composed of mycolic acids and, additionally, it contains several types of non-covalently associated lipids, which, owing to their location, are important players in host–pathogen interaction^{56,57}. Complex lipids synthesized by *M. tuberculosis* include lipoarabinomannan (LAM), phosphatidylinositol mannosides (PIMs), trehalose monomycolates and dimycolates (TMM, TDM/cord factor), diacyl and polyacyl trehaloses (DAT, PAT), phthiocerol dimycocerosates (PDM, DIM, or PDIM), and sulfolipids (SLs)^{56,57}.

PDIMs, for example, have been identified as potent virulence factors of *M. tuberculosis*, as they are required for the induction of phagosomal rupture in the host phagocyte¹³ in conjunction with the ESX-1 system¹⁵ (Figure 1). Moreover, a recent study suggested that *M. tuberculosis* abundantly releases 1-tuberculosinyladenosine (1-TdAb), a secreted lipid that acts as an antacid and lysosomotropic agent⁵⁸. Additionally, comparative genomics between pathogenic *M. tuberculosis* and tubercle bacilli with smooth colony morphology, named *M. canettii*, have uncovered a recombination event in the *pk5* genomic region that has apparently led to the loss of lipooligosaccharides (LOS) in the outer membrane of *M. tuberculosis* during the evolution of the MTBC⁵⁹. This loss of LOS production, which is associated with the appearance of the rough colony morphology

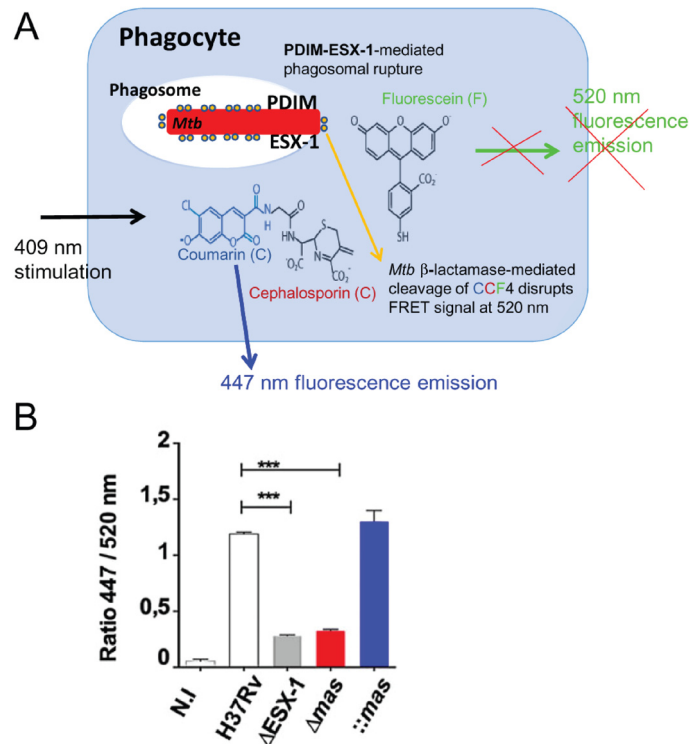


Figure 1. A) Cartoon of the CCF4-based fluorescence resonance energy transfer (FRET) assay in phagocytes infected with ESX-1 and phthiocerol dimycocerosate (PDIM) proficient *Mycobacterium tuberculosis* (*Mtb*) (after⁶⁰). CCF4-AM (Life Technologies) represents a lipophilic, esterified form of the CCF4 substrate, which allows it to readily enter host cells. Endogenous cytoplasmic esterases rapidly convert CCF4-AM into a negatively charged form, which is trapped in the cytosol. Following excitation at 409 nm, a green fluorescence FRET signal (520 nm) is emitted that is changed into a blue fluorescent signal when phagosomal rupture is induced by *Mtb* via its ESX-1 system in cooperation with the virulence lipid PDIM. By this cytosolic contact, mycobacterial β -lactamase (shown as encircled yellow dots linked to the bacterium) gets in contact with the CCF4 and cleaves it, thereby leading to emission of blue fluorescence at ~450 nm (after⁶⁰). **B)** Example of results from ¹⁵ showing the ratios of fluorescence intensities MFI 447 nm/MFI 520 nm of signals obtained by the above-described FRET assay, implicating *Mtb* ESX-1 and PDIM deletion mutants and complemented strains 6 days after infection of THP-1 cells. Image taken from ¹⁵.

in the MTBC members, is thus thought to have contributed to increased fitness and virulence in MTBC members compared to early branching *M. canettii* strains^{59,61}.

The antigen 85 complex

The antigen 85 (Ag85) protein complex consists of a set of secreted fibronectin-binding proteins (Fbps), which are essential for maintaining the integrity of the mycobacterial cell envelope. Namely, Ag85 complex is involved in catalyzing the attachment of mycolic acids to arabinogalactan and the biosynthesis of cord factor. While there are four *fbp* genes (*fbpA–D*) present in the genome of *M. tuberculosis* H37Rv⁶², only three of them, Ag85A–C (FbpA, Rv3804c; FbpB, Rv1886c; and FbpC, Rv0129c), have shown mycoloyltransferase activity in complementation experiments⁶³. The same three Ag85A–C proteins also display an Arg–Arg (RR) secretion signal for the twin arginine translocation (TAT) pathway, known to transport folded proteins through the bacterial inner membrane⁶⁴. Moreover, the Ag85 complex is involved in the invasion of host cells, binding to fibronectin (Fn), tropoelastin, and elastin, essential parts of the extracellular matrix, and, in persistence, preventing maturation of the phagosome^{65–67}.

The strong immunogenicity of the Ag85 proteins has made them privileged targets for vaccine development. Ag85A, for example, was often used as an expression construct for booster vaccines based on attenuated viral vectors such as modified vaccinia virus Ankara (MVA) or adenovirus^{68,69}, although an Ag85A-based booster strategy did not enhance protection in a clinical trial over the protection conferred by BCG⁷⁰. Ag85B was also used as a potential vaccine enhancer. Indeed, a recombinant BCG strain that over-expressed Ag85B induced increased protection in preclinical animal models⁷¹ and was also tested in initial clinical trials⁷². Most interestingly, an increased secretion of Ag85B was also observed in the MTBVAC vaccine candidate, which is currently in clinical evaluation^{73,74}. This attenuated *M. tuberculosis* strain, in which the *phoP* and the *fadD26* genes were deleted in order to attenuate the strain⁷⁵, shows enhanced secretion of Ag85B, which is due to an unexpected PhoP-regulatory loop involving a small ncRNA that modulates the secretion of TAT-secreted proteins, such as Ag85 proteins⁷⁶. In agreement, intraphagocytic release of Ag85B was found to be strongly enhanced in an *M. tuberculosis* PhoP deletion mutant in a dedicated study³⁷ (Figure 2). This example underlines once more that active export and secretion

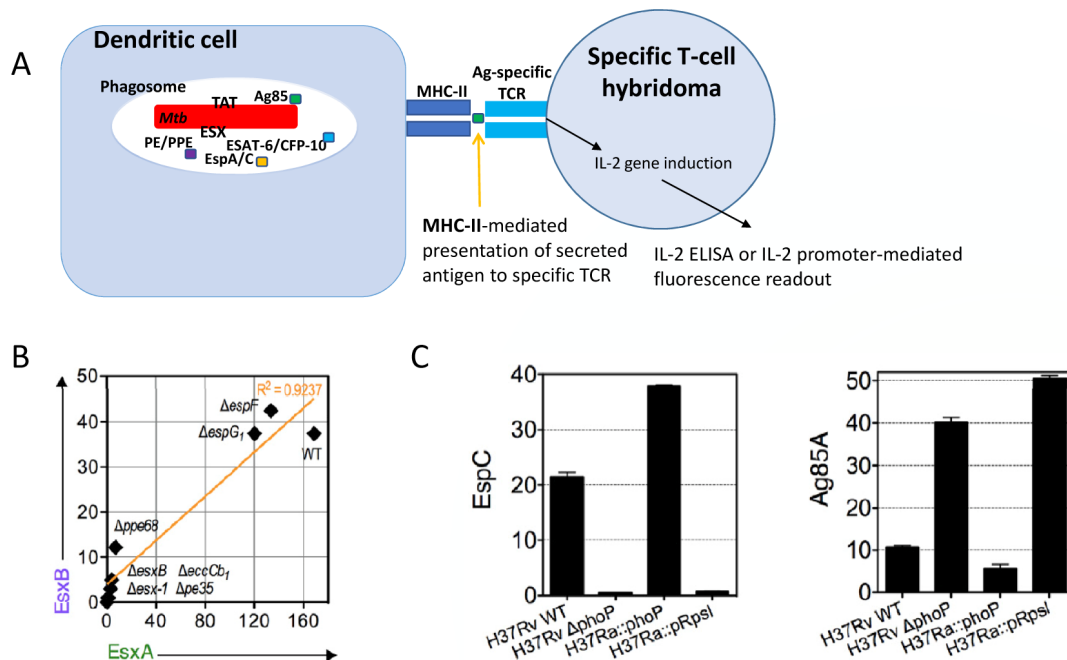


Figure 2. **A**) Cartoon of an intraphagocytic antigen release assay, which uses major histocompatibility complex (MHC)-II-mediated signaling to antigen-specific T-cell hybridoma for the evaluation of secretion of selected proteins as shown for the examples depicted in panels B and C (after³⁷). **B**) Correlation of EsxB (also known as CFP-10) versus EsxA (also known as ESAT-6) intraphagocyte secretion profiles across a panel of various *Mycobacterium tuberculosis* (*Mtb*) wild-type and mutant strains, reused with permission from³⁷. **C**) Example of ESX-1 or twin arginine translocation (TAT) signals obtained by intraphagocytic antigen release assays for *Mtb* H37Rv and *Mtb* H37Ra, wild-type, and mutant strains (image taken from³⁷), which differ in the gene encoding the PhoP two-component virulence regulator⁷⁷ and hence in the secretion profiles of substrates that are secreted by the TAT pathway. The TAT system is regulated by PhoP via a small RNA (Mcr7), as described in⁷⁶. Ag, antigen; CFP-10, 10 kDa Culture Filtrate Protein; ELISA, enzyme-linked immunosorbent assay; ESAT-6, 6 kDa early secretory antigenic

of mycobacterial antigens outside the bacterial cell are crucial for the appropriate recognition of these antigens by the host immune system, a phenomenon that was also observed for ESX antigens^{37,77,78}. This observation also explains in part why heat-killed mycobacterial vaccines have much less immunisation- and protection-capacity than do live attenuated strains, which secrete a wide panel of protective antigens^{79,80}.

Virulence factors expressed during latency/latency antigens

M. tuberculosis is able to switch to a dormant state/latency during stress conditions. In order to design a vaccine also providing protection against latent *M. tuberculosis* infection (LTBI), antigens expressed during latency need to be considered or antigen presentation during latency needs to be stimulated^{4,81}. Zhang *et al.* recently reported that EspI, a protein encoded by *esx-1*, is indeed not essential for ESX-1 secretion during active infection but is responsible for down-regulating ESX-1 secretion during low-energy states in the bacilli. This gives important implications for the role of EspI in latency. An EspI knock-out mutant in long-term infection might be rendered incapable of tuning down the secretion of highly immunogenic ESAT-6 under stress conditions, which might result in a stronger immune response against dormant *M. tuberculosis*⁸².

Host-pathogen interaction, virulence, and immunogenicity

Most successful vaccines act against infectious agents which can be defeated by humoral immune responses, while it has been

proven difficult to design successful vaccines that rely on cellular immune responses, such as in TB⁴. It is undisputed that in TB the CD4⁺ Th1 response is the major factor conferring protection^{1,83–85}. Only fairly recently has evidence emerged that other immune cell subsets such as CD8⁺ T cells, NK cells, Th17 cells, or B cells may provide an important contribution to protection^{4,86–90}. Other factors are well known to play a destructive role, such as Th2 cells and T-regulatory cells/secreted IL-10. Moreover, active TB disease appears to be associated with strong recruitment of neutrophils and type I interferon (IFN) signaling, though the role of type I IFNs in TB remains disputed^{4,91–94}. The innate immune response has a crucial role in protection, especially in early infection control^{95,96}.

ESAT-6 causes phagosomal rupture in the infected host cells, permitting cytosolic contact of the mycobacteria or their secreted products^{18,60,97}. The results of this process are varied and may also have consequences for infection control.

Mycobacterial dsDNA that is likely released because of phagosomal rupture into the cytosol is sensed by absent in melanoma 2 (AIM2), which induces the NLRP3 inflammasome to activate caspase-1, which in turn induces the cleavage of pro-IL-1 β and pro-IL-18 to active, pro-inflammatory IL-1 β and IL-18^{18,98}. Moreover, the link between cytosolic access of mycobacteria and type I IFN signaling has clearly been established. dsDNA in the cytosol activates cyclic GMP-AMP synthase (cGAS), leading to the signaling of the second messenger cGAMP, which

activates the STING pathway (stimulator of IFN genes). The latter is located at the endoplasmic reticulum and triggers Tank-binding kinase 1 (TBK-1), which activates IFN regulatory factor 3 (IRF3), leading to the production of IFN- β (Figure 3). Importantly, cGAMP also activates neighboring cells for the production of type I IFN, augmenting the production of IL-10 and IL-1Ra in these bystander host cells, leading to increased host cell necrosis and tissue damage^{18,94,99–101}. On the other hand, Banks *et al.* recently reported a protective bactericidal effect of IFN- β , activating nitric oxide (NO) production, which has been shown to act in a bactericidal and anti-inflammatory manner, leading to killing of the bacilli and preventing tissue damage⁹⁴. Banks *et al.* suggest a scenario in which *M. tuberculosis* inhibits possibly beneficial autocrine IFN- β signaling while allowing possibly detrimental paracrine IFN- β signaling, while non-pathogenic mycobacteria such as *M. smegmatis* cannot inhibit autocrine IFN- β signaling, which is why the beneficial properties of autocrine type I IFN signaling predominate^{94,102}. It is probable that the role for type I IFN will not be black and white in TB but ambivalent, for example, depending on the timing of signaling during infection (early, late) or the mode of signaling (autocrine, paracrine).

It has been shown that virulent *M. tuberculosis* H37Rv induces necrosis of infected host cells, leading to damage to the mitochondrial inner and outer membrane, and it has been proposed that this could be mediated by ESAT-6, which also leads to the induction of type I IFN by sensing of mitochondrial DNA, while non-virulent H37Ra causes damage only to the outer membrane of host mitochondria, not leading to necrosis¹⁰³. Necrosis is an uncontrolled process of cell death and a destructive process in mycobacterial infection. Meanwhile, apoptosis represents a highly controlled process of cell death. Parts of the apoptotic cell as well as mycobacteria disassemble, safely packaged in apoptotic bodies, and are eventually taken up via efferocytosis by phagocytes. This process prevents tissue damage

and spread of the bacilli and may enhance the activation of naïve professional antigen-presenting cells (APCs), especially dendritic cells (DCs), taking up mycobacterial components. However, this may also lead to infection. The “fate” of the phagocyte is largely dependent on the cocktail of cytokines present, Th1 cytokines being correlates of protection. A Th1-dominated cytokine cocktail produces classically activated macrophages (CAM, M1) and the production of NO, promoting killing of the bacilli and augmenting the Th1 response, while a Th2-dominated cocktail produces alternatively activated macrophages (AAM, M2) that express arginase-1, are impaired in bacterial killing, and augment Th2 responses¹⁰⁴. Moreover, Th1 cytokines stimulate autophagy while Th2 cytokines inhibit autophagy^{103,105–107}. Autophagy has been described as an essential process in the control and clearance of mycobacterial infection, as mice lacking the essential autophagy factor Atg5 are highly susceptible to infection^{108,109}. However, this process may also be entirely due to excess neutrophilic recruitment and resulting tissue damage in Δ Atg5 mice, not to a lack in autophagy, as another study with mice deficient in several players of the autophagy pathway in macrophages, other than Atg5, did not show any higher susceptibility to *M. tuberculosis*^{18,110}. In the latter study, autophagy in macrophages appeared to be insignificant for the outcome of infection with *M. tuberculosis*. However, this finding might have been influenced by the fact that macrophages are not the most important effectors of autophagy compared to DCs, as DCs are needed for the priming of naïve Th1 cells, which eventually produce IFN- γ to activate phagocytes for the clearance of the bacilli¹¹⁰. Evidence has pointed towards a scenario in which ESAT-6 inhibits autophagy at the step of phagosome–lysosome fusion, resulting in reduced secretion of IL-12 in DCs needed for the induction of Th1, which could be restored by rapamycin therapy^{111,112}. However, cytosolic access of mycobacteria may also stimulate autophagy via marking of mycobacterial compounds in the cytosol with ubiquitin for selective autophagy

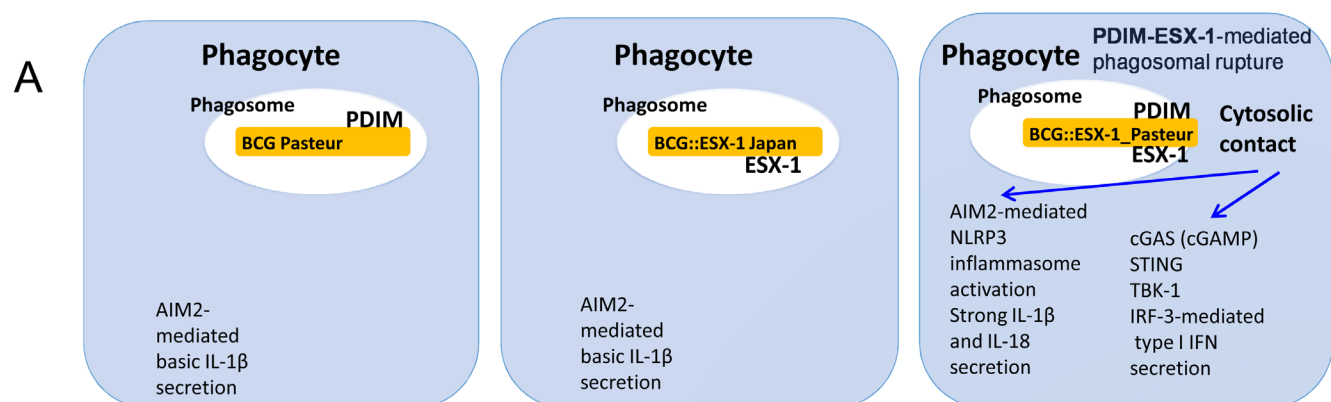


Figure 3. Cartoon showing the impact of the presence of a functional ESX-1 machinery and the outer membrane lipid phthiocerol dimycocerosate (PDIM) on phagosomal rupture for wild-type and recombinant Bacille Calmette–Guérin (BCG) strains, taking into account the results of several recent studies^{15,86,113}. The enhanced activation of cytosolic DNA-mediated innate immune signaling of selected recombinant BCG strains is shown. Cytosolic contact via the cooperation between the ESX-1 secretion system and PDIM is achieved only when both components ESX-1 and PDIM are combined and results in the enhancement of induced CD4⁺ and CD8⁺ T responses, which is correlated with improved protection against a challenge in various preclinical mouse models of infection^{86,113,114}. AIM2, absent in melanoma 2; cGAS, cyclic GMP-AMP synthase; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; STING, stimulator of interferon genes; TBK-1, TANK-binding kinase 1.

or via the AIM2/inflammasome pathway, as seen with the vaccine candidate VPM-1002¹¹⁵. Moreover, cytosolic contact of mycobacteria via the AIM2/inflammasome/IL-18 pathway has been reported as an essential mechanism for the production of IFN- γ by *M. tuberculosis*-independent memory CD8⁺ T cells and NK cells, thus emerging as an important, immediate, and unexpected source of Th1 cytokines⁸⁶.

A recombinant BCG expressing the ESX-1 from *M. tuberculosis* provided improved protection over BCG but, as a downside, was also more virulent in murine models and therefore not an easily usable vaccine candidate^{10,114}. Following this observation, the idea to recombine BCG with the ESX-1 of less-virulent mycobacteria such as *M. marinum* was proposed, which resulted in the construction of a recombinant BCG expressing ESX-1^{Mmar}, which confers improved protection compared to BCG in different murine models. This candidate vaccine induced higher levels of CD4⁺/CD8⁺ T-cell responses, likely through the establishment of cytosolic contact via the heterogeneous ESX-1^{Mmar} system, which induces innate and adaptive signaling events that are not induced by standard BCG strains¹¹³ (Figure 3), while remaining at a similar safety level as parental BCG.

The picture of how exactly ESAT-6 influences intracellular signaling remains an unsolved puzzle. In the context of *M. tuberculosis* infection, ESAT-6 represents a harmful virulence factor, but, in the context of vaccination with recombinant BCG, ESAT-6 acts as a potent immunogen and initiator of important, additional signaling pathways^{114,116}.

For the ESX-5-associated PE/PPE proteins, strong immunogenicity has been demonstrated in murine models, inducing CD4⁺ and CD8⁺ T-cell responses. Importantly, this could also be induced via cross-reactivity by non-ESX-5-associated PE/PPE proteins in a knock-out mutant not expressing the ESX-5-associated PE/PPE, thanks to the redundancy of PE/PPE proteins in the mycobacterial genome and the conservation of the essential ESX-5 secretion system needed for the secretion of a multiplicity of PE/PPE proteins^{43,46}. The exact function of PE/PPE proteins remains largely unknown, but they have been proposed to be involved in mycobacterial capsule/cell-wall integrity, nutrient uptake, antigenic variation, immunodominance, mycobacterial growth in macrophages, and inhibition of phagosome maturation through the induction of phagosomal rupture^{14,43,117,118}. Thus, certain PE/PPE proteins might have an impact on intracellular signaling that is similar to that of ESAT-6/CFP-10 discussed above, while other features such as nutrient uptake may not be such an important factor for immunogenicity but an interesting target to be considered for the attenuation of possible vaccine candidates.

Lipid surface factors such as pathogen-associated molecular patterns play a crucial role during the early phase of infection, aiding the establishment and persistence of infection. They interact with various types of pathogen recognition receptors (PRRs) on phagocytes, which can lead to the priming of phagocytes, the production of chemokines/cytokines for the recruitment of other players of the immune system, and the production of antimicrobial products or can reversely hamper

activation of the phagocyte and mediate the silent entry of the bacilli^{119,120}. One of the most thoroughly studied lipid surface factors is cord factor/TDM, which was first described in the 1950s¹²¹. TDM stimulates PRRs, inducing the production of cytokines and NO, influencing granuloma formation, and playing an important role as an adjuvant; however, it may also be involved in the delay of phagosome maturation^{57,122,123}. Cord factor is abundantly present in all mycobacteria, although subtle differences in mycolate structure seem to play a role in the virulence and pathogenicity of varying mycobacterial species⁵⁷. Other lipid surface factors such as DAT and PAT are associated only with mycobacteria belonging to the MTBC and SL is present only in *M. tuberculosis* strains¹²⁴. Synthesis of DAT, PAT, and SL needs specific polyketide synthase systems (PKSs). DAT and PAT are associated with the delay of phagosome maturation, and SL has been described as a TLR-2 antagonist, which makes PKSs suitable targets for possible attenuation and drugs^{57,125,126}. Other surface lipids have been lost in the MTBC, such as the above-mentioned LOS, because of a recombination event in the *pks5* locus^{59,127}. Interestingly, the lineage 4 strains of the Euro-American lineage of *M. tuberculosis* strains harbor a frameshift mutation in the *pks15/l* gene, which is not present in lineage 2 Beijing strains and in turn leads to the synthesis of phenolic glycolipids (PGLs) in these latter strains^{57,128,129}. This phenomenon has been postulated to be linked with increased virulence of Beijing strains¹³⁰, although other lineage-specific factors also seem to interfere¹³¹. Lipid surface factors thus play a dual role in mycobacteria as immune response boosters, as virulence factors, or even as attenuation factors and could simultaneously be employed as vaccine adjuvants, as drug targets, or for attenuation¹¹⁹. Moreover, lipid surface factors can directly activate the adaptive immune responses via recognition by CD1; the exact role of this alternative activation of T-cells will need to be further elucidated^{132,133}.

Perspectives on vaccine research

There is some skepticism over whether it will be possible to develop a truly effective vaccine against TB, but there is also some evidence that gives hope for a positive outcome. First of all, it is the existence of BCG, which does provide excellent protection against miliary and meningeal TB in children. Second, it is the fact that previous *M. tuberculosis* infection or controlled LTBI does seem to provide some protection against the development of active TB disease after reinfection, as 90–95% of people infected with *M. tuberculosis* exhibit a protective immune response and as a result do not develop active TB in their lifetime¹. In the 1930s, a study from Norway of nurses working in a TB hospital, a high-risk population, showed that nurses with a positive TST prior to their employment at the hospital had a 96% risk reduction of developing active TB compared to nurses who had a negative TST¹³⁴. A review of 18 prospective clinical trials from 2012 confirmed this with a 79% reduced risk of progression to active TB after reinfection in individuals with LTBI compared to previously non-infected people¹³⁵. However, LTBI in itself poses a risk to the development of active TB when control of *M. tuberculosis* is lost, for example because of age or immunosuppression. Moreover, it has been described that the rate of reinfection TB, defined as a recurrent TB episode with a different strain, in

individuals who had previously already developed active TB disease and had successfully been treated, is higher than the rate of new TB cases in the population, suggesting that people who had TB once are at a strongly increased risk of developing TB when reinfected¹³⁶. Indeed, this underlines the variability and importance of individual human immune systems to cope with the infection. One interesting attempt to improve vaccine research is thus to identify correlates of protection by studying the protective immune response in people with LTBI⁵¹. Simultaneously, new vaccine candidates are often designed following two simple principles to either make BCG more immunogenic by introducing strong immunogens such as ESAT-6 or render *M. tuberculosis* attenuated by the deletion of important virulence factors⁴. Given the major advances that have been made recently in the genomic and cellular characterization of virulence factors and virulence strategies of the members of the MTBC and closely related tubercle bacilli, such as *M. canettii*, the challenge is now to transpose this knowledge into practice concerning vaccine research. There are some promising leads, which include a number of recombinant BCG strains that show improved protection in preclinical models^{86,113,137,138}. In addition, some recombinant BCG strains have been or are presently in clinical trials^{72,139}, and very recent results have also suggested that revaccination with BCG has a positive impact against infection with *M. tuberculosis*¹⁴⁰. Alternatively, there is hope that rationally attenuated *M. tuberculosis* strains may induce better protection than standard BCG vaccination, as suggested by different preclinical studies^{46,141}. The fact that the attenuated *M. tuberculosis* strain

MTBVAC is in clinical evaluation is an encouraging sign that attenuated *M. tuberculosis* strains may be part of the future vaccine strategies against *M. tuberculosis* infection and TB disease.

From a more upstream research prospective, the identification of novel virulence factors of pathogenic mycobacteria will certainly continue and might provide new perspectives for vaccine research. Several mycobacterial species that have been considered as most closely related to *M. tuberculosis*, such as *M. marinum* or *M. kansasii*, have been used to identify novel mycobacterial virulence factors^{142–145}. However, according to the most recent genome analyses, several new, recently described mycobacterial species are much more closely related to *M. tuberculosis* than *M. marinum* and *M. kansasii*^{146,147}. Indeed, several virulence factors, such as the fumarate reductase locus, the SL locus, or numerous toxin–antitoxin systems that have been considered as being present only in *M. tuberculosis* and/or *M. canettii*, can also be found in this group of closely related mycobacteria, which constitute a common clade with MTB that was named MTB-associated phylotype (MTBAP)¹⁴⁷. Future research will show if some of these virulence factors might have importance in vaccine research.

Acknowledgements

We are grateful to our many colleagues who were involved in the original work of the subjects reviewed in this article.

References



- World Health Organisation: **Global tuberculosis report 2018**. [Reference Source](#)
- Zhang L, Ru HW, Chen FZ, *et al.*: **Variable Virulence and Efficacy of BCG Vaccine Strains in Mice and Correlation With Genome Polymorphisms**. *Mol Ther.* 2016; **24**(2): 398–405. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Andersen P, Doherty TM: **The success and failure of BCG — implications for a novel tuberculosis vaccine**. *Nat Rev Micro.* 2005; **3**(8): 656–62. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ottenhoff TH, Kaufmann SH: **Vaccines against tuberculosis: Where are we and where do we need to go?** *PLoS Pathog.* 2012; **8**(5): e1002607. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hatherill M, Adams V, Hughes J, *et al.*: **The potential impact of helminth infection on trials of novel tuberculosis vaccines**. *Vaccine.* 2009; **27**(35): 4743–4. [PubMed Abstract](#) | [Publisher Full Text](#)
- Sassetti CM, Boyd DH, Rubin EJ: **Genes required for mycobacterial growth defined by high density mutagenesis**. *Mol Microbiol.* 2003; **48**(1): 77–84. [PubMed Abstract](#) | [Publisher Full Text](#)
-  Griffin JE, Gawronski JD, DeJesus MA, *et al.*: **High-Resolution Phenotypic Profiling Defines Genes Essential for Mycobacterial Growth and Cholesterol Catabolism**. *PLoS Pathog.* 2011; **7**(9): e1002251. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Sassetti CM, Rubin EJ: **Genetic requirements for mycobacterial survival during infection**. *Proc Natl Acad Sci U S A.* 2003; **100**(22): 12989–94. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zhang YJ, Reddy MC, Iorger TR, *et al.*: **Tryptophan Biosynthesis Protects Mycobacteria from CD4 T-Cell-Mediated Killing**. *Cell.* 2013; **155**(6): 1296–308. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pym AS, Brodin P, Brosch R, *et al.*: **Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti***. *Mol Microbiol.* 2002; **46**(3): 709–17. [PubMed Abstract](#) | [Publisher Full Text](#)
- Braunstein M, Espinosa BJ, Chan J, *et al.*: **SecA2 functions in the secretion of superoxide dismutase A and in the virulence of *Mycobacterium tuberculosis***. *Mol Microbiol.* 2003; **48**(2): 453–64. [PubMed Abstract](#) | [Publisher Full Text](#)
- Roberts DM, Liao RP, Wisedchaisri G, *et al.*: **Two sensor kinases contribute to the hypoxic response of *Mycobacterium tuberculosis***. *J Biol Chem.* 2004; **279**(22): 23082–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
-  Quigley J, Hughitt VK, Velikovsky CA, *et al.*: **The Cell Wall Lipid PDIM Contributes to Phagosomal Escape and Host Cell Exit of *Mycobacterium tuberculosis***. *mBio.* 2017; **8**(2): pii: e00148–17. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Bottai D, Di Luca M, Majlessi L, *et al.*: **Disruption of the ESX-5 system of *Mycobacterium tuberculosis* causes loss of PPE protein secretion, reduction of cell wall integrity and strong attenuation**. *Mol Microbiol.* 2012; **83**(6): 1195–209. [PubMed Abstract](#) | [Publisher Full Text](#)
- Augenstein J, Arbues A, Simeone R, *et al.*: **ESX-1 and phthiocerol dimycocerosates of *Mycobacterium tuberculosis* act in concert to cause phagosomal rupture and host cell apoptosis**. *Cell Microbiol.* 2017; **19**(7): e12726. [PubMed Abstract](#) | [Publisher Full Text](#)
- Aguilo N, Gonzalo-Asensio J, Alvarez-Arguedas S, *et al.*: **Reactogenicity to major tuberculosis antigens absent in BCG is linked to improved protection against *Mycobacterium tuberculosis***. *Nat Commun.* 2017; **8**: 16085. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ates LS, Dippenaar A, Ummels R, *et al.*: **Mutations in *ppe38* block PE_{GRS}**



- secretion and increase virulence of *Mycobacterium tuberculosis*. *Nat Microbiol*. 2018; 3(2): 181–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
18. Gröschel MI, Sayes F, Simeone R, *et al.*: **ESX secretion systems: mycobacterial evolution to counter host immunity**. *Nat Rev Micro*. 2016; 14(11): 677–91.
[PubMed Abstract](#) | [Publisher Full Text](#)
 19. Tiwari S, Casey R, Goulding CW, *et al.*: **Infect and Inject: How *Mycobacterium tuberculosis* Exploits Its Major Virulence-Associated Type VII Secretion System, ESX-1**. *Microbiol Spectr*. 2019; 7(3).
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 20. **F** Unnikrishnan M, Constantinidou C, Palmer T, *et al.*: **The Enigmatic Esx Proteins: Looking Beyond Mycobacteria**. *Trends Microbiol*. 2017; 25(3): 192–204.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 21. Green ER, Meccas J: **Bacterial Secretion Systems: An Overview**. *Microbiol Spectr*. 2016; 4(1).
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Mahairas GG, Sabo PJ, Hickey MJ, *et al.*: **Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis***. *J Bacteriol*. 1996; 178(5): 1274–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 23. Hsu T, Hingley-Wilson SM, Chen B, *et al.*: **The primary mechanism of attenuation of bacillus Calmette-Guérin is a loss of secreted lytic function required for invasion of lung interstitial tissue**. *Proc Natl Acad Sci U S A*. 2011; 100(21): 12420–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. Sula L, Radkovský I: **Protective effects of *M. microti* vaccine against tuberculosis**. *J Hyg Epidemiol Microbiol Immunol*. 1976; 20(1): 1–6.
[PubMed Abstract](#)
 25. Hart PD, Sutherland I: **BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life**. *Br Med J*. 1977; 2(6082): 293–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. Houben EN, Bestebroer J, Ummels R, *et al.*: **Composition of the type VII secretion system membrane complex**. *Mol Microbiol*. 2012; 86(2): 472–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
 27. **F** Rosenberg OS, Dovala D, Li X, *et al.*: **Substrates Control Multimerization and Activation of the Multi-Domain ATPase Motor of Type VII Secretion**. *Cell*. 2015; 161(3): 501–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 28. **F** Beckham KS, Ciccarelli L, Bunduc CM, *et al.*: **Structure of the mycobacterial ESX-5 type VII secretion system membrane complex by single-particle analysis**. *Nat Microbiol*. 2017; 2: 17047.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 29. **F** Famelis N, Rivera-Calzada A, Degliesposti G, *et al.*: **Architecture of the mycobacterial type VII secretion system**. *Nature*. 2019.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 30. Renshaw PS, Panagiotidou P, Whelan A, *et al.*: **Conclusive evidence that the major T-cell antigens of the *Mycobacterium tuberculosis* complex ESAT-6 and CFP-10 form a tight, 1:1 complex and characterization of the structural properties of ESAT-6, CFP-10, and the ESAT-6*CFP-10 complex. Implications for pathogenesis and virulence**. *J Biol Chem*. 2002; 277(24): 21598–603.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. Champion PA, Champion MM, Manzanillo P, *et al.*: **ESX-1 secreted virulence factors are recognized by multiple cytosolic AAA ATPases in pathogenic mycobacteria**. *Mol Microbiol*. 2009; 73(5): 950–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 32. Das C, Ghosh TS, Mande SS, *et al.*: **Computational analysis of the ESX-1 region of *Mycobacterium tuberculosis*: insights into the mechanism of type VII secretion system**. *PLoS One*. 2011; 6(11): e27980.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 33. **F** Sala C, Odermatt NT, Soler-Arnedo P, *et al.*: **EspL is essential for virulence and stabilizes EspE, EspF and EspH levels in *Mycobacterium tuberculosis***. *PLoS Pathog*. 2018; 14(12): e1007491.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 34. **F** Lou Y, Rybníček J, Sala C, *et al.*: **EspC forms a filamentous structure in the cell envelope of *Mycobacterium tuberculosis* and impacts ESX-1 secretion**. *Mol Microbiol*. 2017; 103(1): 26–38.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 35. Ates LS, Brosch R: **Discovery of the type VII ESX-1 secretion needle?** *Mol Microbiol*. 2017; 103(1): 7–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 36. Bottai D, Majlessi L, Simeone R, *et al.*: **ESAT-6 secretion-independent impact of ESX-1 genes *espF* and *espG*, on virulence of *Mycobacterium tuberculosis***. *J Infect Dis*. 2011; 203(8): 1155–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Sayes F, Blanc C, Ates LS, *et al.*: **Multiplexed Quantitation of Intraphagocyte *Mycobacterium tuberculosis* Secreted Protein Effectors**. *Cell Rep*. 2018; 23(4): 1072–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 38. Korotkova N, Piton J, Wagner JM, *et al.*: **Structure of EspB, a secreted substrate of the ESX-1 secretion system of *Mycobacterium tuberculosis***. *J Struct Biol*. 2015; 191(2): 236–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Pallen MJ: **The ESAT-6/WXG100 superfamily -- and a new Gram-positive secretion system?** *Trends Microbiol*. 2002; 10(5): 209–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 40. Bitter W, Houben EN, Bottai D, *et al.*: **Systematic genetic nomenclature for type VII secretion systems**. *PLoS Pathog*. 2009; 5(10): e1000507.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 41. Daleke MH, Ummels R, Bawono P, *et al.*: **General secretion signal for the mycobacterial type VII secretion pathway**. *Proc Natl Acad Sci U S A*. 2012; 109(28): 11342–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 42. Daleke MH, van der Woude AD, Parret AH, *et al.*: **Specific chaperones for the type VII protein secretion pathway**. *J Biol Chem*. 2012; 287(38): 31939–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Sayes F, Pawlik A, Frigui W, *et al.*: **CD4⁺ T Cells Recognizing PE/PPE Antigens Directly or via Cross Reactivity Are Protective against Pulmonary *Mycobacterium tuberculosis* Infection**. *PLoS Pathog*. 2016; 12(7): e1005770.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. Gey Van Pittius NC, Gamieldien J, Hide W, *et al.*: **The ESAT-6 gene cluster of *Mycobacterium tuberculosis* and other high G+C Gram-positive bacteria**. *Genome Biol*. 2001; 2(10): RESEARCH0044.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 45. Dumas E, Christina Boritsch E, Vandenbogaert M, *et al.*: **Mycobacterial Pan-Genome Analysis Suggests Important Role of Plasmids in the Radiation of Type VII Secretion Systems**. *Genome Biol Evol*. 2016; 8(2): 387–402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Sayes F, Sun L, Di Luca M, *et al.*: **Strong immunogenicity and cross-reactivity of *Mycobacterium tuberculosis* ESX-5 type VII secretion: Encoded PE-PPE proteins predicts vaccine potential**. *Cell Host Microbe*. 2012; 11(4): 352–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Bottai D, Brosch R: **Mycobacterial PE, PPE and ESX clusters: novel insights into the secretion of these most unusual protein families**. *Mol Microbiol*. 2009; 73(3): 325–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 48. **F** Van Der Meeren O, Hatherill M, Nduba V, *et al.*: **Phase 2b Controlled Trial of M72/AS01_E Vaccine to Prevent Tuberculosis**. *N Engl J Med*. 2018; 379(17): 1621–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 49. Thacher EG, Cavassini M, Audran R, *et al.*: **Safety and immunogenicity of the M72/AS01 candidate tuberculosis vaccine in HIV-infected adults on combination antiretroviral therapy: a phase II/III, randomized trial**. *AIDS*. 2014; 28(12): 1769–81.
[PubMed Abstract](#) | [Publisher Full Text](#)
 50. **F** Kumarasamy N, Poongulali S, Beulah FE, *et al.*: **Long-term safety and immunogenicity of the M72/AS01E candidate tuberculosis vaccine in HIV-positive and -negative Indian adults: Results from a phase II randomized controlled trial**. *Medicine (Baltimore)*. 2018; 97(45): e13120.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 51. Schragar LK, Harris RC, Vekemans J: **Research and development of new tuberculosis vaccines: a review**. *F1000Res*. 2018; 7: 1732.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Ates LS, Sayes F, Frigui W, *et al.*: **RD5-mediated lack of PE_{GRS} and PPE-MPTR export in BCG vaccine strains results in strong reduction of antigenic repertoire but little impact on protection**. *PLoS Pathog*. 2018; 14(6): e1007139.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 53. Baldwin SL, Reese VA, Huang PW, *et al.*: **Protection and Long-Lived Immunity Induced by the ID93/GLA-SE Vaccine Candidate against a Clinical *Mycobacterium tuberculosis* Isolate**. *Clin Vaccine Immunol*. 2016; 23(2): 137–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. **F** Stylianou E, Harrington-Kandt R, Beglov J, *et al.*: **Identification and Evaluation of Novel Protective Antigens for the Development of a Candidate Tuberculosis Subunit Vaccine**. *Infect Immun*. 2018; 86(7): pii: e00014-18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 55. Homolka S, Ubben T, Niemann S: **High Sequence Variability of the ppE18 Gene of Clinical *Mycobacterium tuberculosis* Complex Strains Potentially Impacts Effectivity of Vaccine Candidate M72/AS01E**. *PLoS One*. 2016; 11(3): e0152200.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. **F** Daffé M, Marrakchi H: **Unraveling the Structure of the Mycobacterial Envelope**. *Microbiol Spectr*. 2019; 7(4).
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 57. Madacki J, Mas Fiol G, Brosch R: **Update on the virulence factors of the obligate pathogen *Mycobacterium tuberculosis* and related tuberculosis-causing mycobacteria**. *Infect Genet Evol*. 2019; 72: 67–77.
[PubMed Abstract](#) | [Publisher Full Text](#)
 58. **F** Buter J, Cheng TY, Ghanem M, *et al.*: ***Mycobacterium tuberculosis* releases an anticard that remodels phagosomes**. *Nat Chem Biol*. 2019; 15(9): 889–99.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 59. Boritsch EC, Frigui W, Cascioferro A, *et al.*: ***pks5*-recombination-mediated surface remodelling in *Mycobacterium tuberculosis* emergence**. *Nat Microbiol*. 2016; 1: 15019.
[PubMed Abstract](#) | [Publisher Full Text](#)
 60. **F** Simeone R, Bobard A, Lippmann J, *et al.*: **Phagosomal rupture by**

- Mycobacterium tuberculosis** results in toxicity and host cell death. *PLoS Pathog.* 2012; 8(2): e1002507.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
61. Blouin Y, Cazajous G, Dehan C, *et al.*: Progenitor "Mycobacterium canettii" clone responsible for lymph node tuberculosis epidemic, Djibouti. *Emerging Infect Dis.* 2014; 20(1): 21–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 62. Cole ST, Brosch R, Parkhill J, *et al.*: Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature.* 1998; 393(6685): 537–44.
[PubMed Abstract](#) | [Publisher Full Text](#)
 63. Puech V, Guilhot C, Perez E, *et al.*: Evidence for a partial redundancy of the fibronectin-binding proteins for the transfer of mycoloyl residues onto the cell wall arabinogalactan termini of *Mycobacterium tuberculosis*. *Mol Microbiol.* 2002; 44(4): 1109–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
 64. van Winden VJC, Houben ENG, Braunstein M: Protein Export into and across the Atypical Diderm Cell Envelope of Mycobacteria. *Microbiol Spectr.* 2019; 7(4).
[PubMed Abstract](#) | [Publisher Full Text](#)
 65. Karbalaei Zadeh Babaki M, Soleimanpour S, Rezaee SA: Antigen 85 complex as a powerful *Mycobacterium tuberculosis* immunogene: Biology, immune-pathogenicity, applications in diagnosis, and vaccine design. *Microb Pathog.* 2017; 112: 20–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 66. Belisle JT, Vissa VD, Sievert T, *et al.*: Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science.* 1997; 276(5317): 1420–2.
[PubMed Abstract](#) | [Publisher Full Text](#)
 67. Kuo CJ, Ptak CP, Hsieh CL, *et al.*: Elastin, a novel extracellular matrix protein adhering to mycobacterial antigen 85 complex. *J Biol Chem.* 2013; 288(6): 3886–96.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 68. **F** McShane H: Insights and challenges in tuberculosis vaccine development. *Lancet Respir Med.* 2019; 7(9): 810–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 69. Betts G, Poyntz H, Stylianou E, *et al.*: Optimising immunogenicity with viral vectors: mixing MVA and HAdV-5 expressing the mycobacterial antigen Ag85A in a single injection. *PLoS One.* 2012; 7(12): e50447.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 70. Ndiaye BP, Thienemann F, Ota M, *et al.*: Safety, immunogenicity, and efficacy of the candidate tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med.* 2015; 3(3): 190–200.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 71. Horwitz MA, Harth G, Dillon BJ, *et al.*: Recombinant bacillus calmette-guerin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc Natl Acad Sci U S A.* 2000; 97(25): 13853–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 72. Hoft DF, Blazevic A, Abate G, *et al.*: A new recombinant bacille Calmette-Guérin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers. *J Infect Dis.* 2008; 198(10): 1491–501.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 73. Spertini F, Audran R, Chakour R, *et al.*: Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. *Lancet Respir Med.* 2015; 3(12): 953–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
 74. **F** Tameris M, Mearns H, Penn-Nicholson A, *et al.*: Live-attenuated *Mycobacterium tuberculosis* vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double-blind dose-escalation trial. *Lancet Respir Med.* 2019; 7(9): 757–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 75. **F** Marinova D, Gonzalo-Asensio J, Aguilo N, *et al.*: MTBVAC from discovery to clinical trials in tuberculosis-endemic countries. *Expert Rev Vaccines.* 2017; 16(6): 565–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 76. Solans L, Gonzalo-Asensio J, Sala C, *et al.*: The PhoP-dependent ncRNA Mcr7 modulates the TAT secretion system in *Mycobacterium tuberculosis*. *PLoS Pathog.* 2014; 10(5): e1004183.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 77. Frigui W, Bottai D, Majlessi L, *et al.*: Control of *M. tuberculosis* ESAT-6 secretion and specific T cell recognition by PhoP. *PLoS Pathog.* 2008; 4(2): e33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 78. Majlessi L, Prados-Rosales R, Casadevall A, *et al.*: Release of mycobacterial antigens. *Immunol Rev.* 2015; 264(1): 25–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
 79. Romain F, Augier J, Pescher P, *et al.*: Isolation of a proline-rich mycobacterial protein eliciting delayed-type hypersensitivity reactions only in guinea pigs immunized with living mycobacteria. *Proc Natl Acad Sci U S A.* 1993; 90(11): 5322–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 80. Andersen P: The T cell response to secreted antigens of *Mycobacterium tuberculosis*. *Immunobiology.* 1994; 191(4–5): 537–47.
[PubMed Abstract](#) | [Publisher Full Text](#)
 81. Aagaard C, Hoang T, Dietrich J, *et al.*: A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat Med.* 2011; 17(2): 189–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
 82. Zhang M, Chen JM, Sala C, *et al.*: ESX-1 regulates the ESX-1 secretion system in response to ATP levels in *Mycobacterium tuberculosis*. *Mol Microbiol.* 2014; 93(5): 1057–65.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 83. Oettinger T, Jørgensen M, Ladefoged A, *et al.*: Development of the *Mycobacterium bovis* BCG vaccine: review of the historical and biochemical evidence for a genealogical tree. *Tuber Lung Dis.* 1999; 79(4): 243–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
 84. Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, *et al.*: Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. *Nat Genet.* 2002; 32(1): 97–105.
[PubMed Abstract](#) | [Publisher Full Text](#)
 85. **F** Keane J, Gershon S, Wise RP, *et al.*: Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med.* 2001; 345(15): 1098–104.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 86. **F** Kupz A, Zedler U, Stäber M, *et al.*: ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control in vivo. *J Clin Invest.* 2016; 126(6): 2109–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 87. **F** Khader SA, Bell GK, Pearl JE, *et al.*: IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat Immunol.* 2007; 8(4): 369–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 88. Cooper AM: Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol.* 2009; 27: 393–422.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 89. **F** Schaible UE, Winaw F, Sieling PA, *et al.*: Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat Med.* 2003; 9(8): 1039–46.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 90. Maglione PJ, Chan J: How B cells shape the immune response against *Mycobacterium tuberculosis*. *Eur J Immunol.* 2009; 39(3): 676–86.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 91. **F** Berry MP, Graham CM, McNab FW, *et al.*: An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature.* 2010; 466(7309): 973–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 92. Desvignes L, Wolf AJ, Ernst JD: Dynamic roles of type I and type II IFNs in early infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion. *J Immunol.* 2012; 188(12): 6205–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 93. Stanley SA, Johndrow JE, Manzanillo P, *et al.*: The Type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. *J Immunol.* 2007; 178(5): 3143–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
 94. Banks DA, Ahlbrand SE, Hughitt VK, *et al.*: *Mycobacterium tuberculosis* Inhibits Autocrine Type I IFN Signaling to Increase Intracellular Survival. *J Immunol.* 2019; 202(8): 2348–59.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. Wolf AJ, Desvignes L, Linas B, *et al.*: Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *J Exp Med.* 2008; 205(1): 105–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 96. Day J, Schlesinger LS, Friedman A: Tuberculosis research: going forward with a powerful "translational systems biology" approach. *Tuberculosis (Edinb).* 2010; 90(1): 7–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 97. **F** van der Wel N, Hava D, Houben D, *et al.*: *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell.* 2007; 129(7): 1287–98.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 98. Mishra BB, Moura-Alves P, Sonawane A, *et al.*: *Mycobacterium tuberculosis* protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. *Cell Microbiol.* 2010; 12(8): 1046–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
 99. **F** Manzanillo PS, Shiloh MU, Portnoy DA, *et al.*: *Mycobacterium tuberculosis* activates the DNA-dependent cytosolic surveillance pathway within macrophages. *Cell Host Microbe.* 2012; 11(5): 469–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 100. Vaziri F, Brosch R: ESX/Type VII Secretion Systems-An Important Way Out for Mycobacterial Proteins. *Microbiol Spectr.* 2019; 7(4).
[PubMed Abstract](#) | [Publisher Full Text](#)
 101. **F** Mayer-Barber KD, Andrade BB, Oland SD, *et al.*: Host-directed therapy of

- tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature*. 2014; **511**(7507): 99–103.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
102. **F** Moreira-Teixeira L, Mayer-Barber K, Sher A, *et al.*: **Type I interferons in tuberculosis: Foe and occasionally friend.** *J Exp Med*. 2018; **215**(5): 1273–85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 103. Chen M, Gan H, Remold HG: **A Mechanism of Virulence: Virulent *Mycobacterium tuberculosis* Strain H37Rv, but Not Attenuated H37Ra, Causes Significant Mitochondrial Inner Membrane Disruption in Macrophages Leading to Necrosis.** *J Immunol*. 2006; **176**(6): 3707–16.
[PubMed Abstract](#) | [Publisher Full Text](#)
 104. Wiktorowicz JE, Chowdhury IH, Stafford S, *et al.*: **Integrated Functional Analysis of the Nuclear Proteome of Classically and Alternatively Activated Macrophages.** *Mediators Inflamm*. 2019; **2019**: 3481430.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 105. Lee J, Hartman M, Kornfeld H: **Macrophage apoptosis in tuberculosis.** *Yonsei Med J*. 2009; **50**(1): 1–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 106. Deretic V, Delgado M, Vergne I, *et al.*: **Autophagy in immunity against *mycobacterium tuberculosis*: A model system to dissect immunological roles of autophagy.** *Curr Top Microbiol Immunol*. 2009; **335**: 169–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 107. **F** Martin CJ, Booty MG, Rosebrock TR, *et al.*: **Efferocytosis is an innate antibacterial mechanism.** *Cell Host Microbe*. 2012; **12**(3): 289–300.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 108. **F** Nakagawa I, Amano A, Mizushima N, *et al.*: **Autophagy defends cells against invading group A *Streptococcus*.** *Science*. 2004; **306**(5698): 1037–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 109. **F** Gutierrez MG, Master SS, Singh SB, *et al.*: **Autophagy Is a Defense Mechanism Inhibiting BCG and *Mycobacterium tuberculosis* Survival in Infected Macrophages.** *Cell*. 2004; **119**(6): 753–66.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 110. Kimmey JM, Huynh JP, Weiss LA, *et al.*: **Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection.** *Nature*. 2015; **528**(7583): 565–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 111. Romagnoli A, Etna MP, Giacomini E, *et al.*: **ESX-1 dependent impairment of autophagic flux by *Mycobacterium tuberculosis* in human dendritic cells.** *Autophagy*. 2012; **8**(9): 1357–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 112. Behura A, Mishra A, Chugh S, *et al.*: **ESAT-6 modulates Calcimycin-induced autophagy through microRNA-30a in mycobacteria infected macrophages.** *J Infect*. 2019; **79**(2): 139–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
 113. Gröschel MI, Sayes F, Shin SJ, *et al.*: **Recombinant BCG Expressing ESX-1 of *Mycobacterium marinum* Combines Low Virulence with Cytosolic Immune Signaling and Improved TB Protection.** *Cell Rep*. 2017; **18**(11): 2752–65.
[PubMed Abstract](#) | [Publisher Full Text](#)
 114. Pym AS, Brodin P, Majlessi L, *et al.*: **Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis.** *Nat Med*. 2003; **9**(5): 533–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 115. **F** Watson RO, Manzanillo PS, Cox JS: **Extracellular *M. tuberculosis* DNA Targets Bacteria for Autophagy by Activating the Host DNA-Sensing Pathway.** *Cell*. 2012; **150**(4): 803–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 116. Majlessi L, Brodin P, Brosch R, *et al.*: **Influence of ESAT-6 Secretion System 1 (RD1) of *Mycobacterium tuberculosis* on the Interaction between Mycobacteria and the Host Immune System.** *J Immunol*. 2005; **174**(6): 3570–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 117. Ates LS, Ummels R, Commandeur S, *et al.*: **Essential Role of the ESX-5 Secretion System in Outer Membrane Permeability of Pathogenic Mycobacteria.** *PLoS Genet*. 2015; **11**(5): e1005190.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 118. Ates LS, Houben ENG, Bitter W: **Type VII Secretion: A Highly Versatile Secretion System.** *Microbiol Spectr*. 2016; **4**(1).
[PubMed Abstract](#) | [Publisher Full Text](#)
 119. Dorhoi A, Reece ST, Kaufmann SH: **For better or for worse: The immune response against *Mycobacterium tuberculosis* balances pathology and protection.** *Immunol Rev*. 2011; **240**(1): 235–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
 120. **F** Liu PT, Stenger S, Li H, *et al.*: **Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response.** *Science*. 2006; **311**(5768): 1770–3.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 121. Bloch H, Noll H: **Studies on the virulence of Tubercle bacilli; the effect of cord factor on murine tuberculosis.** *Br J Exp Pathol*. 1955; **36**(1): 8–17.
[PubMed Abstract](#) | [Free Full Text](#)
 122. Indrigo J, Hunter RL Jr, Actor JK: **Cord factor trehalose 6,6'-dimycolate (TDM) mediates trafficking events during mycobacterial infection of murine macrophages.** *Microbiology*. 2003; **149**(Pt 8): 2049–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
 123. **F** Ishikawa E, Ishikawa T, Morita YS, *et al.*: **Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle.** *J Exp Med*. 2009; **206**(13): 2879–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 124. Daffé M, Draper P: **The envelope layers of mycobacteria with reference to their pathogenicity.** *Adv Microb Physiol*. 1998; **39**: 131–203.
[PubMed Abstract](#) | [Publisher Full Text](#)
 125. Passemar C, Arbués A, Malaga W, *et al.*: **Multiple deletions in the polyketide synthase gene repertoire of *Mycobacterium tuberculosis* reveal functional overlap of cell envelope lipids in host-pathogen interactions.** *Cell Microbiol*. 2014; **16**(2): 195–213.
[PubMed Abstract](#) | [Publisher Full Text](#)
 126. **F** Blanc L, Gilleron M, Prandi J, *et al.*: ***Mycobacterium tuberculosis* inhibits human innate immune responses via the production of TLR2 antagonist glycolipids.** *Proc Natl Acad Sci U S A*. 2017; **114**(42): 11205–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 127. Supply P, Marceau M, Manganot S, *et al.*: **Genomic analysis of smooth tubercle bacilli provides insights into ancestry and pathoadaptation of *Mycobacterium tuberculosis*.** *Nat Genet*. 2013; **45**(2): 172–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 128. Constant P, Perez E, Malaga W, *et al.*: **Role of the pks15/1 gene in the biosynthesis of phenolglycolipids in the *Mycobacterium tuberculosis* complex. Evidence that all strains synthesize glycosylated p-hydroxybenzoic methyl esters and that strains devoid of phenolglycolipids harbor a frameshift mutation in the pks15/1 gene.** *J Biol Chem*. 2002; **277**(41): 38148–58.
[PubMed Abstract](#) | [Publisher Full Text](#)
 129. Marmiesse M, Brodin P, Buchrieser C, *et al.*: **Macro-array and bioinformatic analyses reveal mycobacterial 'core' genes, variation in the ESAT-6 gene family and new phylogenetic markers for the *Mycobacterium tuberculosis* complex.** *Microbiology*. 2004; **150**(Pt 2): 483–96.
[PubMed Abstract](#) | [Publisher Full Text](#)
 130. **F** Reed MB, Domenech P, Manca C, *et al.*: **A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response.** *Nature*. 2004; **431**(7004): 84–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 131. Sinsimer D, Huet G, Manca C, *et al.*: **The phenolic glycolipid of *Mycobacterium tuberculosis* differentially modulates the early host cytokine response but does not in itself confer hypervirulence.** *Infect Immun*. 2008; **76**(7): 3027–36.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 132. De Libero G, Singhal A, Lepore M, *et al.*: **Nonclassical T cells and their antigens in tuberculosis.** *Cold Spring Harb Perspect Med*. 2014; **4**(9): a018473.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 133. van Rhijn I, Moody DB: **CD1 and mycobacterial lipids activate human T cells.** *Immunol Rev*. 2015; **264**(1): 138–53.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 134. Heimbeck J: **Incidence of tuberculosis in young adult women, with special reference to employment.** *Brit J Tuberc*. 1938; **32**(3): 154–66.
[Publisher Full Text](#)
 135. Andrews JR, Noubary F, Walensky RP, *et al.*: **Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*.** *Clin Infect Dis*. 2012; **54**(6): 784–91.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 136. Verver S, Warren RM, Beyers N, *et al.*: **Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis.** *Am J Respir Crit Care Med*. 2005; **171**(12): 1430–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
 137. Gengenbacher M, Nieuwenhuizen N, Vogelzang A, *et al.*: **Deletion of nuoG from the Vaccine Candidate *Mycobacterium bovis* BCG AureC::hly Improves Protection against Tuberculosis** *mBio*. 2016; **7**(3): pii: e00679–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 138. Ahn SK, Tran V, Leung A, *et al.*: **Recombinant BCG Overexpressing *phoP-phoR* Confers Enhanced Protection against Tuberculosis.** *Mol Ther*. 2018; **26**(12): 2863–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 139. Grode L, Ganoza CA, Brohm C, *et al.*: **Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial.** *Vaccine*. 2013; **31**(9): 1340–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 140. **F** Nemes E, Geldenhuys H, Rozot V, *et al.*: **Prevention of *M. tuberculosis* Infection with H4:IC31 Vaccine or BCG Revaccination.** *N Engl J Med*. 2018; **379**(2): 138–49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 141. Arbués A, Aguilo JI, Gonzalo-Asensio J, *et al.*: **Construction, characterization and preclinical evaluation of MTBVAC, the first live-attenuated *M. tuberculosis*-based vaccine to enter clinical trials.** *Vaccine*. 2013; **31**(42): 4867–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
 142. Stinear TP, Seemann T, Harrison PF, *et al.*: **Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*.** *Genome Res*. 2008; **18**(5): 729–41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

143.  Cambier CJ, O'Leary SM, O'Sullivan MP, *et al.*: **Phenolic Glycolipid Facilitates Mycobacterial Escape from Microbicidal Tissue-Resident Macrophages.** *Immunity*. 2017; **47**(3): 552–565.e4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
144. Conrad WH, Osman MM, Shanahan JK, *et al.*: **Mycobacterial ESX-1 secretion system mediates host cell lysis through bacterium contact-dependent gross membrane disruptions.** *Proc Natl Acad Sci U S A*. 2017; **114**(6): 1371–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
145. Wang J, McIntosh F, Radomski N, *et al.*: **Insights on the emergence of *Mycobacterium tuberculosis* from the analysis of *Mycobacterium kansasii*.** *Genome Biol Evol*. 2015; **7**(3): 856–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
146.  Tortoli E, Fedrizzi T, Meehan CJ, *et al.*: **The new phylogeny of the genus *Mycobacterium*: The old and the news.** *Infect Genet Evol*. 2017; **56**: 19–25.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
147. Sapriel G, Brosch R: **Shared Pathogenomic Patterns Characterize a New Phylotype, Revealing Transition toward Host-Adaptation Long before Speciation of *Mycobacterium tuberculosis*.** *Genome Biol Evol*. 2019; **11**(8): 2420–38.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:




Editorial Note on the Review Process

F1000 Faculty Reviews are written by members of the prestigious F1000 Faculty. They are commissioned and are peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

- 1 **Manuel Elkin Patarroyo**
Pathology Department, Faculty of Medicine, Universidad Nacional de Colombia, Carrera, Colombia
Competing Interests: No competing interests were disclosed.
- 2 **Jun Liu**
Department of Molecular Genetics, University of Toronto, Toronto, Canada
Competing Interests: No competing interests were disclosed.
- 3 **Ramandeep Singh**
Tuberculosis Research Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Haryana, India
Competing Interests: No competing interests were disclosed.
- 4 **Helen Fletcher** 
Tuberculosis Centre, London School of Hygiene and Tropical Medicine, London, UK
Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research