

# Breaching the phagosome, the case of the tuberculosis agent

Roxane Simeone, Fadel Sayes, Emeline Lawarée, Roland Brosch

► **To cite this version:**

Roxane Simeone, Fadel Sayes, Emeline Lawarée, Roland Brosch. Breaching the phagosome, the case of the tuberculosis agent. Cellular Microbiology, Wiley, 2021, 23 (7), pp.e13344. 10.1111/cmi.13344 .  
pasteur-03413281

**HAL Id: pasteur-03413281**

**<https://hal-pasteur.archives-ouvertes.fr/pasteur-03413281>**

Submitted on 5 Nov 2021

**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.



1 Breaching the phagosome, the case of the tuberculosis agent

2

3 (Phagosome/invasion dynamics)

4

5 Roxane Simeone\*, Fadel Sayes\*, Emeline Lawaree\* and Roland Brosch

6

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

8 France;

9

10 \*Contributed equally to this review article;

11

12 Corresponding author: Roland Brosch, [roland.brosch@pasteur.fr](mailto:roland.brosch@pasteur.fr)

13

14

## 15 **Abstract**

16 The interactions between microbes and their hosts are among the most complex biological  
17 phenomena known today. The interaction may reach from overall beneficial interaction, as  
18 observed for most microbiome/microbiota related interactions to interaction with virulent  
19 pathogens, against which host cells have evolved sophisticated defense strategies. Amongst  
20 the latter, the confinement of invading pathogens in a phagosome plays a key role, which  
21 often results in the destruction of the invader, whereas some pathogens may counteract  
22 phagosomal arrest and survive by gaining access to the cytosol of the host cell. In the current  
23 review we will discuss recent insights into this dynamic process of host-pathogen interaction,  
24 using *Mycobacterium tuberculosis* and related pathogenic mycobacteria as main examples.

## 26 **Take Aways**

- 27 • Phagosomal rupture is a key event in mycobacterial host-pathogen interaction
- 28 • Combined action of protein and lipid virulence factors induce phagosomal rupture
- 29 • Mycobacteria-induced phagosomal rupture leads to enhanced intracellular signalling
- 30 • Egress of mycobacteria or their products to the cytosol is linked to host cell death

## 32 **Introduction**

33 In this review dedicated to recent research on bacteria-induced phagosomal rupture, our  
34 discussion will mainly focus on *Mycobacterium tuberculosis* (*Mtb*), the causative agent of  
35 human tuberculosis (TB). This key human pathogen was only relatively recently counted  
36 among the pathogens that have the ability to rupture the phagosome and egress to the cytosol  
37 of host phagocytes. A short comparative analysis of *Mtb* with selected classical cytosolic  
38 pathogens of other bacterial phyla will complete the presented perspectives.

## 40 **Factors of *Mtb* that enable phagosomal rupture:**

41 *Mtb* represents a bacterial pathogen that has caused billions of deaths in the history of  
42 mankind, and tuberculosis remains a leading cause of death in many countries today. Despite  
43 numerous studies that have aimed during many years to elucidate the actions that *Mtb* is using  
44 to circumvent host defenses, our knowledge on these features is still limited. Thus, it is not  
45 surprising that novel aspects linked to the infection mechanisms of *Mtb* are identified more or  
46 less regularly, even more than 130 years after the discovery of the tubercle bacillus. One such  
47 recently revealed aspect that has changed our perception of mycobacterial infection biology

48 was the discovery of a concerted action between well-known virulence lipids and equally  
49 well-known virulence proteins of *Mtb*, acting together to induce phagosomal rupture in  
50 phagocytes (J Augenstreich et al., 2017; J. Augenstreich et al., 2020; Barczak et al., 2017).

51 Indeed, *Mtb* produces a large variety of unique and complex lipids (Daffe &  
52 Marrakchi, 2019), which contribute to the physical resistance of *Mtb* and its interaction with  
53 the host. Briefly, *Mtb*'s envelope has 4 parts: (1) a plasma membrane, which is mainly  
54 composed of phospholipids; (2) a layer of peptidoglycan covalently linked to a layer of  
55 arabinogalactan; (3) a mycomembrane, also called mycobacterial outer membrane, which has  
56 an inner and an outer leaflet, whereby the inner leaflet is composed of mycolic acids that are  
57 esterified with the arabinogalactan layer and the outer leaflet is composed of a variety of non-  
58 covalently linked lipids, such as phthiocerol dimycocerosates (abbreviated as DIM or PDIM),  
59 trehalose dimycolates (TDMs) and sulfolipids (SL) that are involved in the interaction  
60 between *Mtb* and host cells; (4) at the outermost part, a capsule layer is present, which is  
61 formed by a matrix of glucan, proteins, (lipo)polysaccharides and small amounts of lipids.  
62 The example of DIM/PDIM being involved in phagosomal rupture is thus one new aspect of  
63 the previously defined functions of mycobacterial lipids that contribute to the virulence of  
64 *Mtb* (Camacho, Ensergueix, Perez, Gicquel, & Guilhot, 1999; Cox, Chen, McNeil, & Jacobs,  
65 1999).

66 Apart from various lipids, the cell envelope of *Mtb* also contains numerous protein  
67 secretion systems, which are usually embedded within the plasma membrane, and play  
68 important roles in host pathogen interaction. Among them are the Sec-dependent general  
69 secretory pathway (Feltcher, Sullivan, & Braunstein, 2010), the Twin Arginine Translocation  
70 (TAT) system (Palmer & Berks, 2012; Solans et al., 2014) and the ESX/Type VII Secretion  
71 Systems (T7SS) (Abdallah et al., 2007; Chirakos, Balaram, Conrad, & Champion, 2020;  
72 Groschel, Sayes, Simeone, Majlessi, & Brosch, 2016).

73 While the genome of *Mtb* (Cole et al., 1998) encodes five ESX T7SS (ESX-1 to ESX-  
74 5), in the current review, we will mainly focus on the ESX-1 system, which is absent from  
75 live attenuated vaccine strains *Mycobacterium bovis* BCG (BCG) (Hsu et al., 2003; Pym,  
76 Brodin, Brosch, Huerre, & Cole, 2002) and *Mycobacterium microti* M.P. Prague (Orgeur et  
77 al., 2021), and its role in processes leading to induction of phagosomal rupture. The most  
78 well-known ESX-1 substrate in this context is EsxA (also known as Early Secretory  
79 Antigenic Target of 6 kDa, ESAT-6) (Andersen, Andersen, Sorensen, & Nagai, 1995;  
80 Sorensen, Nagai, Houen, Andersen, & Andersen, 1995), which is required for full virulence  
81 of *Mtb* and the related fish pathogen *Mycobacterium marinum* (reviewed in (Groschel et al.,

82 2016) and (Chirakos et al., 2020), respectively). Moreover, a study on clinical isolates of *Mtb*  
83 identified one strain harboring a frameshift mutation that inhibited EsxA secretion, which led  
84 to an attenuation of virulence in both macrophage and mouse infection models and also  
85 caused a decrease of pro-inflammatory cytokine release (Clemmensen et al., 2017).

86 Concerning the predicted functional implications of EsxA in virulence, the first  
87 membranolytic activity of EsxA was described by the use of purified EsxA protein that  
88 caused lysis of artificial lipids bilayers (Hsu et al., 2003). These studies were followed by  
89 demonstration of membranolytic activity of EsxA on liposome preparations, representing  
90 phospholipid compositions typical for eukaryotic membranes (de Jonge et al., 2007). Notably,  
91 this study also tested an EsxA preparation that was extracted directly from *Mtb*. Several other  
92 studies have focused on the EsxA membrane lysis activity by comparing the activities of  
93 EsxA proteins from pathogenic and non-pathogenic mycobacteria (De Leon et al., 2012; Peng  
94 & Sun, 2016) or by generating specific point mutations in order to disrupt the membrane lytic  
95 activity of EsxA (Ma, Keil, & Sun, 2015). However, the membranolytic activity of EsxA,  
96 produced in *Escherichia coli*, has been reconsidered based on recent *in vitro* studies, which  
97 found that traces of detergent ASB-14 remained attached to purified ESAT-6 and were  
98 responsible for the lytic activity of these preparations, even if proteinase K treatment removed  
99 EsxA from the sample (Conrad et al., 2017; Refai et al., 2015). While the ASB-14-mediated  
100 lytic activity of EsxA preparations that used this detergent in the purification protocol, were  
101 recently confirmed by independent experiments (J. Augenstreich et al., 2020), such studies  
102 also showed that EsxA produced without the use of ASB-14 still showed membranolytic  
103 activity (Aguilera et al., 2020; J. Augenstreich et al., 2020). These results are in good  
104 agreement with results obtained from lytic assays using native EsxA preparations from *Mtb*  
105 that did not use ASB-14 in the purification protocol (J. Augenstreich et al., 2020; de Jonge et  
106 al., 2007). Taken together, it seems that some confusion on the lytic activity of certain EsxA  
107 preparations were caused by particular purification protocols, which, however, have been  
108 resolved in recent studies by the use of EsxA preparations that were prepared without ASB-14  
109 and had retained membranolytic activity. From these latter experiments it seems clear that  
110 EsxA itself does show intrinsic membranolytic activity, which is in good agreement with  
111 results from *in vivo* experiments that show loss of biological activity in case of certain  
112 attenuating mutations or truncation of EsxA (Brodin et al., 2005).

113 The interaction between *Mtb* and host cells is a combination of strategic steps from both sides  
114 that have been selected during long-lasting coevolution (Bottai et al., 2020; Ngabonziza et al.,  
115 2020; Queval, Brosch, & Simeone, 2017). *Mtb* is phagocytosed by macrophages and interferes

116 with phagolysosome maturation by blocking the phagosomal acidification in order to survive  
117 inside host cells (Lugo-Villarino & Neyrolles, 2014; C.J. Queval et al., 2017). Since the  
118 seminal work of Armstrong and Hart in 1971, the intracellular localization of *Mtb* inside host  
119 cells was thought to be exclusively within the phagosome (Armstrong & Hart, 1971).  
120 Although this view was challenged by selected electron microscopy (EM)-based studies  
121 (Leake, Myrvik, & Wright, 1984; McDonough, Kress, & Bloom, 1993; Myrvik, Leake, &  
122 Wright, 1984), it was mainly the use of sophisticated cryo-immunogold EM, which finally  
123 questioned the previously widely-accepted hypothesis of the exclusive intracellular  
124 localization of *Mtb* (Houben et al., 2012; van der Wel et al., 2007). These results suggested  
125 that *Mtb* is able to gain access to the cytosol three days post-infection and that this  
126 phenomenon was ESX-1-dependent (Houben et al., 2012; van der Wel et al., 2007). Indeed,  
127 several research groups using independent techniques confirmed and extended these initial  
128 findings, and established that a functional ESX-1 system was fundamental for *Mtb* to rupture  
129 the phagosome and gain access the cytosol of phagocytes. Wong and Jacobs used monoclonal  
130 antibodies against Galectin-3 and ubiquitinated proteins for identification of damaged  
131 phagosomal-membranes in *Mtb*-infected THP-1 cells (Wong & Jacobs, 2011). As initially  
132 shown in the context of *Shigella* and *Listeria* infections, intracellular galectin-3 accumulates  
133 in structures in vicinity of bacteria that lyse the phagocytic vacuole (Paz et al., 2010).  
134 Similarly, an assay that was based on  $\beta$ -lactamase-activated interruption of a Fluorescence  
135 Resonance Energy Transfer (FRET) signal has been successfully adapted for identification of  
136 phagosomal rupture and contact of *Mtb* with the cytosol of host cells (Simeone et al., 2012;  
137 Simeone et al., 2015). In such a FRET assay, after phagocytosis, *Mtb*-infected phagocytes are  
138 loaded with a FRET-inducing probe that is sensitive to cleavage by  $\beta$ -lactamase activity,  
139 which stems from bacteria that have established cytosolic contact (Simeone, Majlessi,  
140 Enninga, & Brosch, 2016). As an initial readout for this approach, fluorescent microscopy  
141 was employed (Simeone et al., 2012), and then the assay was adapted to flowcytometry as  
142 readout (Simeone et al., 2015). Both methods confirmed that interruption of the FRET signal,  
143 visible as a change from green to blue fluorescence, was only obtained when phagocytes were  
144 infected with *Mtb* strains harboring an intact ESX-1 system, while infections with the  
145 naturally ESX-1-deficient BCG vaccine strains did not induce changes in the fluorescence  
146 type, thereby indicating that BCG strains are unable to rupture the phagosome and to establish  
147 cytosolic contact, unless they are complemented with a functional ESX-1 system from *Mtb*  
148 (Simeone et al., 2012), or *M. marinum* (Groschel et al., 2017).

149           However, as briefly mentioned above, a functional ESX-1 system is not the only  
150 requirement for *Mtb* to rupture the phagosome. Recent studies from independent groups,  
151 established that the ability of *Mtb* to gain access to the cytosol also required the production  
152 and export of the virulence lipids DIM/PDIM (J Augenstreich et al., 2017; Lerner et al., 2017;  
153 Lerner et al., 2020; Quigley et al., 2017). Indeed, these extractable lipids have been shown to  
154 play important roles in host-pathogen interaction, such as the arrest of phagosomal  
155 acidification (Astarie-Dequeker et al., 2009) and they are also involved in inducing host cell  
156 death (Passemar et al., 2014). Most recently, employment of different techniques in parallel,  
157 including the labelling of galectin-3 and ubiquitinated proteins for the identification of  
158 damaged phagosomal membranes in infected macrophages and a FRET-based  
159 cytofluorometric approach for the detection of the induction of phagosomal rupture, first  
160 revealed that (i) DIM/PDIM are involved in the induction of the phagosomal rupture and (ii)  
161 that both EsxA/ESX-1 and DIM/PDIMs were required to induce phagosomal rupture and  
162 membrane damage in *Mtb*-infected phagocytes (J Augenstreich et al., 2017). Moreover, it was  
163 found that complementation of BCG strains with the ESX-1 system from *Mtb* only enabled  
164 DIM/PDIM-producing BCG substrains to induce changes in the FRET signals, whereas ESX-  
165 1 complementation of BCG strains that had lost the ability to produce and/or secrete  
166 DIM/PDIMs, (e.g. BCG Japan or BCG Moreau (Chen, Islam, Ren, & Liu, 2007)), did not  
167 induce changes in the FRET signals (J Augenstreich et al., 2017). The significance of  
168 DIM/PDIM in the process of phagosomal rupture was independently confirmed by a study  
169 that used an *Mtb* strain bearing a transposon insertion in the *mmpL7* gene, which encodes a  
170 transporter of DIM/PDIM across the bacterial plasma-membrane to the mycomembrane  
171 (Camacho et al., 1999). It was found that infection of macrophages with the *MmpL7*-ko *Mtb*  
172 strain showed much less induction of phagosomal damage compared to cells infected the *Mtb*  
173 *MmpL7* WT strain (Quigley et al., 2017). Moreover, in a study that used a non-redundant *Mtb*  
174 transposon mutant library, high-content imaging and a multiparametric analysis for evaluating  
175 pathogen and host phenotypes, it was observed that mutants in the DIM/PDIM and ESX-1  
176 pathways both showed similar profiles of impaired intracellular survival and reduced  
177 induction of type I interferon responses, suggesting a concerted impact of both pathways on  
178 phagosomal damage and cytosolic signaling (Barczak et al., 2017). Finally, DIM/PDIM have  
179 also been shown to facilitate cytosolic access of *Mtb* in human lymphatic endothelial cells  
180 (hLEC), as evaluated by electron microscopy (Lerner et al., 2018). Taken together,  
181 convergent data from experiments done by different research groups and by using different

182 methods indicate that phagosomal rupture induced by *Mtb* is an ESX-1 and DIM/PIDM-  
183 dependent process, which can be observed in various cellular infection models.

184 The ability to gain access to the cytosol by phagosomal rupture is a key process for  
185 *Mtb* that likely has been selected during evolution in order to circumvent host defenses and  
186 get access to nutrients during infection (Simeone et al., 2016). However, it is also clear that  
187 cellular mechanisms exist that try to prevent or repair such phagosomal damage. Earlier  
188 studies showed that the Endosomal Sorting Complex Required for Transport (ESCRT)  
189 machinery is involved in repair of phagosomal membranes (Jimenez et al., 2014). Indeed, the  
190 ESCRT machinery has been reported to be recruited to *Mtb*-containing phagosomes in an  
191 ESX1-dependent manner (Mittal et al., 2018), thereby regulating inflammation and cell  
192 viability (Beckwith et al., 2020) (Figure 1). These findings suggest a dynamic interaction  
193 between bacterial factors that induce phagosomal rupture and host factors that may repair or  
194 prevent this process. Similar observations have also been made for *M. marinum* during  
195 infection of the social amoeba *Dictyostelium discoideum* (López-Jiménez et al., 2018).

196 Most interestingly, *Mtb*-induced phagosomal rupture has also been observed under *in*  
197 *vivo* conditions, using a mouse model that included an enrichment of infected phagocytes and  
198 a screen for tracking these rare cells, which were characterized by carrying a CD45.1  
199 hematopoietic allelic marker (Simeone et al., 2015). In the same study, it was also shown that  
200 inhibition of phagosomal acidification intensified phagosomal rupture in *Mtb*-infected  
201 phagocytes, which pointed to the importance of the immune system and certain host  
202 resistance factors, such as the natural resistance-associated macrophage protein (Nramp)-1, in  
203 the control of *Mtb*-induced phagosomal damage (Simeone et al., 2015). Strong impact of  
204 immune control on the frequency of phagosomal rupture during *in vivo* infections with *Mtb*  
205 was also reported in a recent pre-print showing results from a collaborative project led by the  
206 team of N. van der Wel (van der Niet et al., 2020). In this study, which was focused on the  
207 analysis by electron microscopy of samples from mycobacteria-infected human- and animal-  
208 tissues, only low numbers of cytosolic bacilli were found in mouse-, zebrafish-, armadillo-  
209 and patient tissues infected with *Mtb*, *M. marinum* or *Mycobacterium leprae*, respectively. In  
210 contrast, when innate or adaptive immunity was compromised, as in SCID or IL-1R1-  
211 deficient mice, a larger amount of cytosolic *Mtb* bacilli were detected in lungs of infected  
212 mice, suggesting that the cytosolic localization of mycobacteria *in vivo* is controlled by  
213 adaptive immune responses and selected host resistance factors (van der Niet et al., 2020).

214 Indeed, phagosomal rupture has also a strong impact on host cell signaling and host  
215 immune responses, which will be discussed in the following section of the review.



216

217 **Impact of phagosomal rupture on host signaling**

218 As discussed briefly above, mycobacteria-induced phagosomal membrane damage is an ESX-  
219 1 and DIM/PDIM-dependent mechanism, which triggers various host intracellular signaling  
220 pathways, leading to cytokine production, inflammasome activation and autophagy that  
221 profoundly influence cell fate and the outcome of infection (Bussi & Gutierrez, 2019; Chai,  
222 Wang, Liu, & Ge, 2020; Groschel et al., 2016; Kroesen, Madacki, Frigui, Sayes, & Brosch,  
223 2019).

224 It has been shown that cytosolic release of dsDNA inside infected host macrophages is  
225 recognized as pathogen-associated molecular pattern (PAMP) by the host innate cytosolic  
226 sensor AIM2 (Absent in Melanoma 2). This event likely represents the first crucial step in the  
227 activation of the NLRP3 (nucleotide-binding oligomerization domain (NOD)-like receptor  
228 pyrin domain-containing-3)-dependent inflammasome activation cascade that then leads to  
229 caspase-1 activity, which cleaves the inactive pro-IL-1 $\beta$  and pro-IL-18 cytokine precursors  
230 into the active cytokines IL-1 $\beta$  and IL-18, respectively (Dorhoi et al., 2012; Mishra et al.,  
231 2010). IL-1 $\beta$  is a key innate pro-inflammatory cytokine that plays a central protective role  
232 during infection with mycobacteria (Bussi & Gutierrez, 2019; Fremond et al., 2007).  
233 Moreover, ESX-1-dependent NLRP3 inflammasome activation and subsequent IL-18  
234 secretion in CD11c<sup>+</sup> cells were also found to stimulate noncognate IFN- $\gamma$  production by *Mtb*  
235 antigen-independent memory CD8<sup>+</sup> T cells and NK cells, conferring an additional level of  
236 early protective immune responses against TB in mice (Kupz et al., 2016). This process does  
237 not seem to be restricted to infection with *Mtb*, but was also reported for infection with the  
238 emerging mycobacterial pathogen *Mycobacterium abscessus* (Kim, Kim, Kook, & Kim,  
239 2020), whereby bacteria with rough colony morphology type increased mitochondrial ROS  
240 and increased release of oxidized mitochondrial DNA into the cytosol of murine  
241 macrophages, resulting in enhanced NLRP3 inflammasome-mediated IL-1 $\beta$  and cGAS-  
242 STING-dependent IFN-I production (Kim et al., 2020). This finding is in agreement with  
243 results from a study using a high-density transposon screen, which found the involvement of  
244 the *M. abscessus* ESX-4 type VII secretion system in intracellular growth and cytosolic  
245 access of *M. abscessus* (Laencina et al., 2018). In this regard it is particularly noteworthy that  
246 the fast-growing *M. abscessus* does not harbor an ESX-1 secretion system, but encodes an  
247 EccE4 protein, which is missing from most other mycobacterial ESX-4 secretion systems  
248 (Dumas et al., 2016). Finally, it also should be emphasized that IL-1R1-deficient mice show  
249 more cytosolic *Mtb* than fully immunocompetent mice in their lungs (van der Niet et al.,

250 2020), suggesting that ESX-1-induced phagosomal rupture and cytosolic localization of *Mtb*  
251 is counteracted by immune responses of the IL-1 cytokine / receptor families.

252 In parallel to the NLRP3-mediated signaling pathway, ESX-1 mediated cytosolic  
253 release of dsDNA also plays an important role for the cGAS–STING pathway. Several  
254 independent studies showed that transfer of dsDNA to the host cytosol induced the synthesis  
255 of cyclic GMP-AMP (cGAMP), a second messenger that interacts with STING (stimulator of  
256 IFN genes) (Collins et al., 2015; Wassermann et al., 2015; Watson et al., 2015). This process  
257 activates TBK-1 (tank-binding kinase 1) and triggers the STING/TBK-1/IRF3 signaling  
258 pathway, leading to IFN- $\beta$  production and transcription of a subset of interferon-stimulated  
259 genes, including IP-10 and IL-10 (Ablasser & Chen, 2019; Dey et al., 2015; Kroesen et al.,  
260 2019; Majlessi & Brosch, 2015; Manzanillo, Shiloh, Portnoy, & Cox, 2012) (Figure 1).

261 Several studies that used murine models or human cellular models highlighted  
262 immune modulating and anti-inflammatory properties of IFN- $\beta$ , or other type I IFNs (Cooper  
263 & Khader, 2008; Moreira-Teixeira et al., 2020; Novikov et al., 2011). However, the  
264 contributing role of type I IFN in the protection against virulent *Mtb* during the early and late  
265 phases of infection is not fully understood, as type I IFN release might under some  
266 circumstances also counteract protective IL-1 $\beta$  responses (Desvignes, Wolf, & Ernst, 2012;  
267 Manca et al., 2001). From a practical perspective, the integration of a virulence-neutral ESX-1  
268 secretion system, originating from *M. marinum*, into the *M. bovis* BCG genetic background,  
269 which induced IFN- $\beta$  release and higher IL-1 $\beta$  responses, also significantly increased the  
270 protective efficacy of this recombinant BCG::Esx-1<sup>Mmar</sup> vaccine candidate in different murine  
271 models, as compared to the BCG parental strain (Groschel et al., 2017).

272 Apart from DNA-induced signaling pathways, it was recently also reported that  
273 mycobacterial RNA is released into the infected macrophage cytosol in SecA2- and ESX-1-  
274 dependent manner (Cheng & Schorey, 2018), thereby triggering IFN- $\beta$  production through a  
275 RIG-1 (retinoic acid-inducible gene-1) mediated pathway and a cross-talk between DNA and  
276 RNA sensor pathways (Cheng & Schorey, 2018) (Figure 1). Taken together, it is clear that  
277 gaining access to the cytosol during mycobacterial infection is a key process that determines a  
278 cascade of innate and adaptive immune responses that decide whether the pathogen or the  
279 host will “win” the control of the infection process.

280

### 281 **Phagosomal rupture and autophagy induction**

282 In addition to the above described effects of phagosomal rupture on innate and adaptive  
283 immunity processes, another important cell biological process, known as selective autophagy

284 pathway, is linked to the detection of phagosome-derived cytosolic DNA, involving the  
285 cGAS/STING/TBK-1 signaling cascade. This process is a powerful host defense mechanism  
286 to control intracellular pathogens and usually plays a beneficial role in anti-mycobacterial  
287 innate and adaptive immunity (Krakauer, 2019; Xiao & Cai, 2020). Knockdown of cGAS in  
288 human or mouse macrophages blocks cytokine production and induction of *Mtb*-induced  
289 selective autophagy pathway (Collins et al., 2015; Watson et al., 2015), whereas TBK-1 acts  
290 as a pivotal regulator of host innate immune control of mycobacterial growth (Pilli et al.,  
291 2012). Recognition of cytosolic dsDNA by the STING cytosolic pathway was also required  
292 for targeting bacteria with LC3, p62 and NDP52 ubiquitin adaptors, leading to their delivery  
293 to autophagosomes and restricted mycobacterial replication (Watson et al., 2015; Watson,  
294 Manzanillo, & Cox, 2012). In the earlier study, it was shown that mouse cells missing the  
295 autophagy protein 5 (Atg5) were highly susceptible to infection with *Mtb*, which led to the  
296 assumption that Atg5-mediated autophagy was an important mechanism to help control  
297 mycobacterial infections, similar to findings by Castillo and colleagues (Castillo et al., 2012).  
298 In a parallel study, cytosolic access of mycobacteria was found essential for the accumulation  
299 of the lipidated autophagosome-associated isoform of LC3 (LC3-II) in dendritic cells  
300 (Romagnoli et al., 2012). Moreover, for IFN- $\gamma$ -activated macrophages, it was demonstrated  
301 that the host protein ubiquilin 1 promoted IFN- $\gamma$ -mediated autophagic clearance of *Mtb*  
302 (Sakowski et al., 2015). Similarly, another study reported that ubiquitin binds to an *Mtb* PE-  
303 PGRS surface protein, Rv1468c, and directly recruits autophagy receptor p62 to deliver  
304 mycobacteria into LC3-associated autophagosomes, thereby contributing to mycobacterial  
305 clearance (Chai et al., 2019). A study using *Mtb*-infected primary murine macrophages  
306 revealed that a particular autophagy receptor, TAX1BP1, mediates clearance of  
307 ubiquitinated *Mtb* and targets bacteria to LC3-positive phagophores (Budzik et al., 2020).  
308 By using mass spectrometry and bio-informatics analyses, the authors identified hundreds of  
309 dynamically regulated phosphorylation and ubiquitylation sites, suggesting that dramatic  
310 remodeling of multiple host pathways occurred during infection with *Mtb* (Budzik et al.,  
311 2020). In a parallel study which used human induced pluripotent stem cell-derived  
312 macrophages (iPSDMs) it was found that upon ESX-1-mediated phagosomal rupture, *Mtb*  
313 induced the formation of LC3B-positive tubulovesicular autophagosomes (Bernard et al.,  
314 2020).

315 Taken together, all these studies suggest that autophagy is an important innate immune  
316 mechanism against pathogenic mycobacteria. Nevertheless, it should be mentioned that the  
317 role of certain proteins, such as Atg5, which are often considered as being mainly involved in

318 the autophagy pathway, might also have other cellular functions. For example, a study using  
319 Atg5-deficient mice as model has suggested that the increased susceptibility of Atg5-deficient  
320 mice to infection with *Mtb* was due to altered neutrophil recruitment and associated immune  
321 system pathologies, rather than the impairment of autophagy (Kimmey et al., 2015).

322

### 323 **Impact of phagosomal rupture on cell death**

324 Phagosomal rupture represents an important molecular event in mycobacteria-infected  
325 macrophages, which can have severe consequences for the infecting bacterium and/or the host  
326 macrophage. Apart from the damage of the vacuolar compartment caused by ESX-1  
327 proficient *Mtb*, a subsequent damage of the host cell plasma membrane has also been  
328 observed, which caused K<sup>+</sup> efflux and activation of NLRP3-dependent IL-1 $\beta$  release and  
329 pyroptosis, facilitating the spread of *Mtb* to neighbouring cells (Beckwith et al., 2020). These  
330 observations are in agreement with previous data, that showed substantially increased cell  
331 death caused by ESX-1 proficient *Mtb* strains, compared to *Mtb* strains secreting truncated  
332 ESX-1 protein(s) (Simeone et al., 2012). Finally, it should also be mentioned that a recent  
333 paper reported that type I interferon signaling mediates *Mtb*-induced macrophage death  
334 (Zhang, Jiang, Pfau, Ling, & Nathan, 2021), which adds additional possibilities to other  
335 reported virulence-enhancing consequences of type I interferon release (Moreira-Teixeira,  
336 Mayer-Barber, Sher, & O'Garra, 2018; Moreira-Teixeira et al., 2020).

337

### 338 **How can this knowledge be used for vaccine development ?**

339 What can we learn from mycobacteria-induced phagosomal rupture and the involved  
340 cytosolic sensing pathways for developing new generations of more protective anti-TB  
341 vaccines, able to activate host cytosolic surveillance pathway and trigger optimal innate and  
342 adaptive immune responses ?

343 In this regard, as briefly mentioned above, we recently elaborated a low-virulent rBCG  
344 Pasteur::ESX-1<sup>Mmar</sup> vaccine strain, which heterologously expresses the *esx-1* region of *M.*  
345 *marinum*, and thereby is able to induce phagosomal rupture and cGAS/STING/TBK-1/IRF3  
346 cytosolic recognition in the host (Groschel et al., 2017). This strain induces IFN- $\beta$  production  
347 and enhanced activation of AIM-2/NLRP3 inflammasome, representing innate immune  
348 signaling pathways that mimic the natural infection with virulent *Mtb*, resulting in higher  
349 initiation of mycobacteria-specific CD8<sup>+</sup> T cell immunity and a higher proportion of  
350 polyfunctional CD4<sup>+</sup> Th1 effectors specific to ESX-1 antigens. These features of rBCG::ESX-

351 1<sup>Mmar</sup> provided superior protection in murine aerosol challenge models, compared to parental  
352 BCG, which used various highly virulent *Mtb* strains (Groschel et al., 2017).

353 The *Mtb*  $\Delta$ pe25-pe19 strain (Bottai et al., 2012) is another live-attenuated anti-TB  
354 vaccine candidate that elicits ESX-1-mediated cytosolic immune signaling, due to its  
355 functional ESX-1 type VII secretion system (Bottai et al., 2012; Sayes et al., 2016; Sayes et  
356 al., 2012). This strain efficiently activates innate and adaptive immune responses comparable  
357 to virulent *Mtb*, including mycobacteria-specific effector memory CD4<sup>+</sup> T cells, while  
358 showing strong attenuation in cellular and *in vivo* infection models (Bottai et al., 2012; Sayes  
359 et al., 2012). Its use as an attenuated live vaccine candidate displayed significant improved  
360 TB protection efficacy in selected mouse models relative to BCG (Sayes et al., 2016; Sayes et  
361 al., 2012).

362 In conclusion, it seems well that breaching the phagosome represents an important  
363 feature for mycobacterial pathogens to evade destruction by the host cell, which can be  
364 exploited by specially engineered mycobacterial live vaccine strains to induce beneficial  
365 innate and adaptive immune responses that cannot be induced by standard BCG vaccination.  
366 The construction and use of ESX-1-proficient attenuated mycobacterial strains thus represent  
367 a promising strategy for the design of a new generations of anti-TB vaccine candidates.

368

### 369 **Comparison of mycobacterial phagosomal rupture to other bacteria**

370 Cytoplasmic access is a widely used strategy of bacterial pathogens that occurs in  
371 professional phagocytes and in invaded epithelial or endothelial cells. Phagosomal rupture  
372 and escape following secretion of bacterial effectors is not restricted to *Mtb*, as most cytosolic  
373 bacterial pathogens have adopted selected strategies of entry into the host cytosol (Pizarro-  
374 Cerdá, Charbit, Enninga, Lafont, & Cossart, 2016). In the following paragraph we will  
375 present a few selected examples, but we also would like to emphasize that there are many  
376 more cytosolic bacterial pathogens existing that would merit further description, which  
377 however is beyond the scope of the current review.

378 One of the best characterized examples of phagosomal rupture concerns the Gram-  
379 positive opportunistic pathogen *Listeria monocytogenes* that escapes from the vacuole *via* the  
380 action of listeriolysin O (LLO) and type C phospholipases (PLC), both secreted by the Sec  
381 pathway (Burg-Golani et al., 2013; Renier, Micheau, Talon, Hébraud, & Desvaux, 2012).  
382 LLO, a cholesterol-dependent cytolysin, binds cholesterol from the vacuolar membrane and  
383 forms large pores that prevent acidification of phagosomes in macrophages (Burg-Golani et  
384 al., 2013; Cossart, 2011). This process delays phagosome-lysosome fusion prior the

385 destruction of the vacuole membrane, which is finally achieved with the help of a PLC that  
386 directly hydrolyzes phospholipids from the membrane (Gilbert, 2010; Goldfine, Johnston, &  
387 Knob, 1993). Interestingly, *Mtb* also encodes up to four PLCs, which however, do not seem to  
388 be implicated in the phagosomal rupture process induced by *Mtb*. While a first study  
389 suggested that PLCs from *Mtb* might play a role in virulence in a mouse infection model  
390 (Raynaud et al., 2002), a latter study that used the same mutant strains and additional  
391 constructs did not find an attenuated virulence profile for PLC-deletion mutants of *Mtb* in a  
392 murine infection model (Le Chevalier et al., 2015). Moreover, the PLC-deletion mutants  
393 remained fully capable to induce phagosomal rupture in the widely used THP-1 cellular  
394 infection model, arguing that *Mtb* might use a different molecular mechanism to rupture  
395 phagosomal membranes than *L. monocytogenes* (Le Chevalier et al., 2015). Indeed, as  
396 described further above, *Mtb* requires an intact ESX-1 secretion system as well as export of  
397 PDIM virulence lipids for inducing phagosomal rupture and gaining access to the cytosol of  
398 the host macrophage (J Augenstein et al., 2017). As for other Gram-positive bacteria,  
399 *Staphylococcus aureus* has been described to reach the host cytoplasm by escaping from  
400 phagosomes via the release of cytolytic peptides such as phenol-soluble modulins (Grosz et  
401 al., 2014).

402         Among Gram-negative bacteria, *Shigella flexneri* is one of the most studied examples  
403 that gains access to the host cytosol. Indeed, upon internalization into the host cell, *S. flexneri*  
404 ruptures the vacuole membrane within 10 minutes (Paz et al., 2010), following the type III  
405 secretion system (T3SS)-dependent insertion of the effectors/translocators IpaB and IpaC into  
406 the vacuole membrane (Du et al., 2016; Picking et al., 2005; Russo, Duncan, Wiscovitch,  
407 Hachey, & Goldberg, 2019). In a similar way than LLO, IpaB displays haemolytic activity  
408 (High, Mounier, Prévost, & Sansonetti, 1992). Once inserted into the membrane, IpaB  
409 tetramers form a discrete ion channel that permits inflows and outflows of small molecules,  
410 leading to phagosomal membrane rupture and bacterial escape (Dickenson et al., 2013).

411         While mainly extracellular, a recent study has demonstrated that *Pseudomonas*  
412 *aeruginosa* also actively replicates inside macrophages (Kroken, Chen, Evans, Yahr, &  
413 Fleiszig, 2018), and escapes from the phagosome in a T3SS dependent manner (Garai, Berry,  
414 Moussouni, Bleves, & Blanc-Potard, 2019).

415         Phagosomal escape is also essential for *Francisella tularensis* intracellular replication  
416 within the host and depends entirely on secretion of the so-called pathogenicity determinant  
417 proteins PdpC and PdpD via the type VI secretion system (T6SS), known to deliver bacterial  
418 effectors across both bacterial and eukaryotic cells (Brodmann, Dreier, Broz, & Basler, 2017).

419 The exact molecular mechanism employed by PdpC and PdpD to rupture the phagosomal  
420 membrane remains to be elucidated. However, it is known that these two proteins are encoded  
421 within the *Francisella* Pathogenicity Island (FPI), but do not share similarity with known  
422 bacterial effectors or pore forming toxins. This finding is reminiscent to some extent of the  
423 low level of mechanistic insights that are available for the ESX-1- and PDIM-mediated  
424 phagosomal rupture processes induced by *Mtb* and related mycobacteria, which are still under  
425 discussion (J Augenstreich et al., 2017; J. Augenstreich et al., 2020; Conrad et al., 2017; de  
426 Jonge et al., 2007; De Leon et al., 2012).

427 Finally, it is interesting to underline that some intracellular bacteria do not trigger  
428 phagosomal escape and prevent phagosome-lysosome fusion by secreting dedicated bacterial  
429 effectors, which help them to create a replication-permissive vacuole within the infected host.  
430 Indeed, in both *Brucella* spp. and *Legionella* spp., the bacterial effectors are secreted by the  
431 type IV secretion system (T4SS) or the Dot/Icm T4SS, respectively (Atluri, Xavier, de Jong,  
432 den Hartigh, & Tsolis, 2011; Isberg, O'Connor, & Heidtman, 2009), further demonstrating the  
433 importance of specialized secretion systems for bacterial replication within the host.

434

### 435 **Conclusions**

436 From recent years' research it has become more and more evident that mycobacteria-induced  
437 phagosomal rupture represents a key event in pathogen-host interaction. While for many years  
438 it was thought that *Mtb* exclusively replicated inside vacuoles, convergent immunological and  
439 cell biological data point to periods during infection when *Mtb* gains access to the cytosol of  
440 host phagocytes. Despite intense research in the last years this process still holds many  
441 secrets, but which step by step are being elucidated, as was for example recently shown for  
442 the infection process inside selected *in vivo* models (Simeone et al., 2015; van der Niet et al.,  
443 2020). Given the novel tools that are progressively becoming available for detailed  
444 observations of the nanomachines involved in host-pathogen interaction (Lawarée &  
445 Custódio, 2019), deeper insights into mechanistic details of the interaction between  
446 mycobacterial pathogens and host cells shall be obtained in the near future. Research linked to  
447 phagosomal rupture induced by pathogenic mycobacteria will thus remain an exciting subject,  
448 whose new insights might also help to find new intervention strategies against a pathogen that  
449 continues to threaten the health and lives of millions of people.

450

### 451 **Acknowledgements**

452 We are grateful to our many colleagues who were involved in the original work of the  
453 subjects reviewed in this article. Related work in our laboratory was supported in part by the  
454 French National Research Council ANR (ANR-10-LABX-62-IBEID, ANR-16-CE35-0009,  
455 ANR-16-CE15-0003), and the European Union's Horizon 2020 Research and Innovation  
456 Program (643381).

457

## 458 **References**

- 459 Abdallah, A. M., Gey van Pittius, N. C., Champion, P. A., Cox, J., Luirink, J.,  
460 Vandenbroucke-Grauls, C. M., . . . Bitter, W. (2007). Type VII secretion system of  
461 mycobacteria show the way. *Nat Rev Microbiol*, 5(11), 883-891. doi:nrmicro1773  
462 [pii]10.1038/nrmicro1773
- 463 Ablasser, A., & Chen, Z. J. (2019). cGAS in action: Expanding roles in immunity and  
464 inflammation. *Science*, 363(6431). doi:10.1126/science.aat8657
- 465 Aguilera, J., Karki, C. B., Li, L., Vazquez Reyes, S., Estevo, I., Grajeda, B. I., . . . Sun, J.  
466 (2020). N ( $\alpha$ -Acetylation of the virulence factor EsxA is required for mycobacterial  
467 cytosolic translocation and virulence. *J Biol Chem*, 295(17), 5785-5794.  
468 doi:10.1074/jbc.RA119.012497
- 469 Andersen, P., Andersen, A. B., Sorensen, A. L., & Nagai, S. (1995). Recall of long-lived  
470 immunity to *Mycobacterium tuberculosis* infection in mice. *J Immunol*, 154(7), 3359-  
471 3372.
- 472 Armstrong, J. A., & Hart, P. D. (1971). Response of cultured macrophages to *Mycobacterium*  
473 *tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J Exp Med.*,  
474 134(3 Pt 1), 713-740.
- 475 Astarie-Dequeker, C., Le Guyader, L., Malaga, W., Seaphanh, F. K., Chalut, C., Lopez, A., &  
476 Guilhot, C. (2009). Phthiocerol dimycocerosates of *M. tuberculosis* participate in  
477 macrophage invasion by inducing changes in the organization of plasma membrane  
478 lipids. *PLoS Pathog.*, 5(2), e1000289. doi: 1000210.1001371/journal.ppat.1000289.  
479 Epub 1002009 Feb 1000286.
- 480 Atluri, V. L., Xavier, M. N., de Jong, M. F., den Hartigh, A. B., & Tsolis, R. M. (2011).  
481 Interactions of the human pathogenic *Brucella* species with their hosts. *Annu Rev*  
482 *Microbiol*, 65, 523-541. doi:10.1146/annurev-micro-090110-102905
- 483 Augenstreich, J., Arbues, A., Simeone, R., Haanappel, E., Wegener, A., Sayes, F., . . .  
484 Astarie-Dequeker, C. (2017). ESX-1 and phthiocerol dimycocerosates of  
485 *Mycobacterium tuberculosis* act in concert to cause phagosomal rupture and host cell  
486 apoptosis. *Cell Microbiol*, 19, e12726. doi:DOI:10.1111/cmi.12726
- 487 Augenstreich, J., Haanappel, E., Sayes, F., Simeone, R., Guillet, V., Mazeret, S., . . . Astarie-  
488 Dequeker, C. (2020). Phthiocerol Dimycocerosates From *Mycobacterium tuberculosis*  
489 Increase the Membrane Activity of Bacterial Effectors and Host Receptors. *Front Cell*  
490 *Infect Microbiol*, 10, 420. doi:10.3389/fcimb.2020.00420
- 491 Barczak, A. K., Avraham, R., Singh, S., Luo, S. S., Zhang, W. R., Bray, M. A., . . . Hung, D.  
492 T. (2017). Systematic, multiparametric analysis of *Mycobacterium tuberculosis*  
493 intracellular infection offers insight into coordinated virulence. *PLoS Pathog.*, 13(5),  
494 e1006363. doi: 1006310.1001371/journal.ppat.1006363. eCollection 1002017 May.
- 495 Beckwith, K. S., Beckwith, M. S., Ullmann, S., Sætra, R. S., Kim, H., Marstad, A., . . . Flo, T.  
496 H. (2020). Plasma membrane damage causes NLRP3 activation and pyroptosis during  
497 *Mycobacterium tuberculosis* infection. *Nat Commun*, 11(1), 2270.  
498 doi:10.1038/s41467-020-16143-6



499 Bernard, E. M., Fearn, A., Bussi, C., Santucci, P., Peddie, C. J., Lai, R. J., . . . Gutierrez, M.  
500 G. (2020). M. tuberculosis infection of human iPSC-derived macrophages reveals  
501 complex membrane dynamics during xenophagy evasion. *J Cell Sci*, *134*(5).  
502 doi:10.1242/jcs.252973

503 Bottai, D., Di Luca, M., Majlessi, L., Frigui, W., Simeone, R., Sayes, F., . . . Esin, S. (2012).  
504 Disruption of the ESX-5 system of Mycobacterium tuberculosis causes loss of PPE  
505 protein secretion, reduction of cell wall integrity and strong attenuation. *Mol*  
506 *Microbiol*, *83*(6), 1195-1209.

507 Bottai, D., Frigui, W., Sayes, F., Di Luca, M., Spadoni, D., Pawlik, A., . . . Brosch, R. (2020).  
508 TbD1 deletion as a driver of the evolutionary success of modern epidemic  
509 Mycobacterium tuberculosis lineages. *Nat Commun.*, *11*(1), 684. doi:  
510 10.1038/s41467-41020-14508-41465.

511 Brodin, P., de Jonge, M. I., Majlessi, L., Leclerc, C., Nilges, M., Cole, S. T., & Brosch, R.  
512 (2005). Functional analysis of early secreted antigenic target-6, the dominant T-cell  
513 antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion,  
514 complex formation, virulence, and immunogenicity. *J Biol Chem*, *280*(40), 33953-  
515 33959.

516 Brodmann, M., Dreier, R. F., Broz, P., & Basler, M. (2017). Francisella requires dynamic  
517 type VI secretion system and ClpB to deliver effectors for phagosomal escape. *Nat*  
518 *Commun*, *8*, 15853. doi:10.1038/ncomms15853

519 Budzik, J. M., Swaney, D. L., Jimenez-Morales, D., Johnson, J. R., Garelis, N. E., Repasy, T.,  
520 . . . Cox, J. S. (2020). Dynamic post-translational modification profiling of  
521 Mycobacterium tuberculosis-infected primary macrophages. *Elife*, *9*.  
522 doi:10.7554/eLife.51461

523 Burg-Golani, T., Pozniak, Y., Rabinovich, L., Sigal, N., Nir Paz, R., & Herskovits, A. A.  
524 (2013). Membrane chaperone SecDF plays a role in the secretion of Listeria  
525 monocytogenes major virulence factors. *J Bacteriol*, *195*(23), 5262-5272.  
526 doi:10.1128/jb.00697-13

527 Bussi, C., & Gutierrez, M. G. (2019). Mycobacterium tuberculosis infection of host cells in  
528 space and time. *FEMS Microbiol Rev*, *43*(4), 341-361. doi:10.1093/femsre/fuz006

529 Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B., & Guilhot, C. (1999). Identification  
530 of a virulence gene cluster of Mycobacterium tuberculosis by signature-tagged  
531 transposon mutagenesis. *Mol. Microbiol.*, *34*, 257-267.

532 Castillo, E. F., Dekonenko, A., Arko-Mensah, J., Mandell, M. A., Dupont, N., Jiang, S., . . .  
533 Deretic, V. (2012). Autophagy protects against active tuberculosis by suppressing  
534 bacterial burden and inflammation. *Proc Natl Acad Sci U S A*, *109*(46), E3168-3176.  
535 doi:10.1073/pnas.1210500109

536 Chai, Q., Wang, L., Liu, C. H., & Ge, B. (2020). New insights into the evasion of host innate  
537 immunity by Mycobacterium tuberculosis. *Cell Mol Immunol*, *17*(9), 901-913.  
538 doi:10.1038/s41423-020-0502-z

539 Chai, Q., Wang, X., Qiang, L., Zhang, Y., Ge, P., Lu, Z., . . . Liu, C. H. (2019). A  
540 Mycobacterium tuberculosis surface protein recruits ubiquitin to trigger host  
541 xenophagy. *Nat Commun*, *10*(1), 1973. doi:10.1038/s41467-019-09955-8

542 Chen, J. M., Islam, S. T., Ren, H., & Liu, J. (2007). Differential productions of lipid virulence  
543 factors among BCG vaccine strains and implications on BCG safety. *Vaccine*, *25*(48),  
544 8114-8122. doi:S0264-410X(07)01077-8 [pii]10.1016/j.vaccine.2007.09.041

545 Cheng, Y., & Schorey, J. S. (2018). Mycobacterium tuberculosis-induced IFN- $\beta$  production  
546 requires cytosolic DNA and RNA sensing pathways. *J Exp Med*, *215*(11), 2919-2935.  
547 doi:10.1084/jem.20180508

548 Chirakos, A. E., Balaram, A., Conrad, W., & Champion, P. A. (2020). Modeling Tubercular  
549 ESX-1 Secretion Using Mycobacterium marinum. *Microbiol Mol Biol Rev*, 84(4).  
550 doi:10.1128/membr.00082-19

551 Clemmensen, H. S., Knudsen, N. P. H., Rasmussen, E. M., Winkler, J., Rosenkrands, I.,  
552 Ahmad, A., . . . Aagaard, C. (2017). An attenuated Mycobacterium tuberculosis  
553 clinical strain with a defect in ESX-1 secretion induces minimal host immune  
554 responses and pathology. *Sci Rep*, 7, 46666. doi:10.1038/srep46666

555 Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., . . . Barrell, B. G.  
556 (1998). Deciphering the biology of Mycobacterium tuberculosis from the complete  
557 genome sequence. *Nature*, 393(6685), 537-544.

558 Collins, A. C., Cai, H., Li, T., Franco, L. H., Li, X. D., Nair, V. R., . . . Shiloh, M. U. (2015).  
559 Cyclic GMP-AMP Synthase Is an Innate Immune DNA Sensor for Mycobacterium  
560 tuberculosis. *Cell Host Microbe*, 17(6), 820-828. doi:10.1016/j.chom.2015.05.005

561 Conrad, W. H., Osman, M. M., Shanahan, J. K., Chu, F., Takaki, K. K., Cameron, J., . . .  
562 Ramakrishnan, L. (2017). Mycobacterial ESX-1 secretion system mediates host cell  
563 lysis through bacterium contact-dependent gross membrane disruptions. *Proc Natl  
564 Acad Sci U S A*, 114(6), 1371–1376.

565 Cooper, A. M., & Khader, S. A. (2008). The role of cytokines in the initiation, expansion, and  
566 control of cellular immunity to tuberculosis. *Immunol Rev*, 226, 191-204.  
567 doi:10.1111/j.1600-065X.2008.00702.x

568 Cossart, P. (2011). Illuminating the landscape of host-pathogen interactions with the  
569 bacterium *Listeria monocytogenes*. *Proc Natl Acad Sci U S A*, 108(49), 19484-19491.  
570 doi: 19410.11073/pnas.1112371108. Epub 1112372011 Nov 1112371123.

571 Cox, J. S., Chen, B., McNeil, M., & Jacobs, W. R., Jr. (1999). Complex lipid determines  
572 tissue-specific replication of Mycobacterium tuberculosis in mice. *Nature*, 402(6757),  
573 79-83.

574 Daffe, M., & Marrakchi, H. (2019). Unraveling the Structure of the Mycobacterial Envelope.  
575 *Microbiol Spectr.*, 7(4).(doi), 10.1128/microbiolspec.GPP1123-0027-2018.

576 de Jonge, M. I., Pehau-Arnaudet, G., Fretz, M. M., Romain, F., Bottai, D., Brodin, P., . . .  
577 Brosch, R. (2007). ESAT-6 from Mycobacterium tuberculosis dissociates from its  
578 putative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing  
579 activity. *J Bacteriol.* , 189, 6028-6034.

580 De Leon, J., Jiang, G., Ma, Y., Rubin, E., Fortune, S., & Sun, J. (2012). Mycobacterium  
581 tuberculosis ESAT-6 exhibits a unique membrane-interacting activity that is not found  
582 in its ortholog from non-pathogenic Mycobacterium smegmatis. *J Biol Chem*, 287(53),  
583 44184-44191. doi:M112.420869 [pii]10.1074/jbc.M112.420869

584 Desvignes, L., Wolf, A. J., & Ernst, J. D. (2012). Dynamic roles of type I and type II IFNs in  
585 early infection with Mycobacterium tuberculosis. *J Immunol*, 188(12), 6205-6215.  
586 doi:10.4049/jimmunol.1200255

587 Dey, B., Dey, R. J., Cheung, L. S., Pokkali, S., Guo, H., Lee, J. H., & Bishai, W. R. (2015). A  
588 bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates  
589 innate resistance to tuberculosis. *Nat Med*, 21(4), 401-406. doi:10.1038/nm.3813

590 Dickenson, N. E., Choudhari, S. P., Adam, P. R., Kramer, R. M., Joshi, S. B., Middaugh, C.  
591 R., . . . Picking, W. D. (2013). Oligomeric states of the Shigella translocator protein  
592 IpaB provide structural insights into formation of the type III secretion translocon.  
593 *Protein Sci*, 22(5), 614-627. doi:10.1002/pro.2245

594 Dorhoi, A., Nouailles, G., Jorg, S., Hagens, K., Heinemann, E., Pradl, L., . . . Kaufmann, S.  
595 H. E. (2012). Activation of the NLRP3 inflammasome by Mycobacterium tuberculosis  
596 is uncoupled from susceptibility to active tuberculosis. *European Journal of  
597 Immunology*, 42(2), 374-384.

598 Du, J., Reeves, A. Z., Klein, J. A., Twedt, D. J., Knodler, L. A., & Lesser, C. F. (2016). The  
599 type III secretion system apparatus determines the intracellular niche of bacterial  
600 pathogens. *Proc Natl Acad Sci U S A*, *113*(17), 4794-4799.  
601 doi:10.1073/pnas.1520699113

602 Dumas, E., Boritsch, E. C., Vandenbergert, M., Rodriguez de la Vega, R. C., Thiberge, J. M.,  
603 Caro, V., . . . Sapriel, G. (2016). Mycobacterial pan-genome analysis suggests  
604 important role of plasmids in the radiation of type VII secretion systems. *Genome Biol*  
605 *Evol*, *8*(2), 387-402. doi:10.1093/gbe/evw001.

606 Feltcher, M. E., Sullivan, J. T., & Braunstein, M. (2010). Protein export systems of  
607 *Mycobacterium tuberculosis*: novel targets for drug development? *Future Microbiol*,  
608 *5*(10), 1581-1597. doi:10.2217/fmb.10.112

609 Fremont, C. M., Togbe, D., Doz, E., Rose, S., Vasseur, V., Maillet, I., . . . Quesniaux, V. F.  
610 (2007). IL-1 receptor-mediated signal is an essential component of MyD88-dependent  
611 innate response to *Mycobacterium tuberculosis* infection. *J Immunol*, *179*(2), 1178-  
612 1189.

613 Garai, P., Berry, L., Moussouni, M., Bleves, S., & Blanc-Potard, A. B. (2019). Killing from  
614 the inside: Intracellular role of T3SS in the fate of *Pseudomonas aeruginosa* within  
615 macrophages revealed by *mgtC* and *oprF* mutants. *PLoS Pathog*, *15*(6), e1007812.  
616 doi:10.1371/journal.ppat.1007812

617 Gilbert, R. J. (2010). Cholesterol-dependent cytolysins. *Adv Exp Med Biol*, *677*, 56-66.  
618 doi:10.1007/978-1-4419-6327-7\_5

619 Goldfine, H., Johnston, N. C., & Knob, C. (1993). Nonspecific phospholipase C of *Listeria*  
620 *monocytogenes*: activity on phospholipids in Triton X-100-mixed micelles and in  
621 biological membranes. *J Bacteriol*, *175*(14), 4298-4306. doi:10.1128/jb.175.14.4298-  
622 4306.1993

623 Groschel, M. I., Sayes, F., Shin, S. J., Frigui, W., Pawlik, A., Orgeur, M., . . . Brosch, R.  
624 (2017). Recombinant BCG Expressing ESX-1 of *Mycobacterium marinum* Combines  
625 Low Virulence with Cytosolic Immune Signaling and Improved TB Protection. *Cell*  
626 *Rep.*, *18*(11), 2752-2765. doi: 2710.1016/j.celrep.2017.2702.2057.

627 Groschel, M. I., Sayes, F., Simeone, R., Majlessi, L., & Brosch, R. (2016). ESX secretion  
628 systems: mycobacterial evolution to counter host immunity. *Nat Rev Microbiol.*,  
629 *14*(11), 677-691. doi: 610.1038/nrmicro.2016.1131. Epub 2016 Sep 1026.

630 Grosz, M., Kolter, J., Paprotka, K., Winkler, A. C., Schäfer, D., Chatterjee, S. S., . . .  
631 Fraunholz, M. (2014). Cytoplasmic replication of *Staphylococcus aureus* upon  
632 phagosomal escape triggered by phenol-soluble modulins. *Cell Microbiol*, *16*(4),  
633 451-465. doi:10.1111/cmi.12233

634 High, N., Mounier, J., Prévost, M. C., & Sansonetti, P. J. (1992). IpaB of *Shigella flexneri*  
635 causes entry into epithelial cells and escape from the phagocytic vacuole. *The EMBO*  
636 *Journal*, *11*(5), 1991-1999.

637 Houben, D., Demangel, C., van Ingen, J., Perez, J., Baldeon, L., Abdallah, A. M., . . . Peters,  
638 P. J. (2012). ESX-1-mediated translocation to the cytosol controls virulence of  
639 mycobacteria. *Cell Microbiol*, *14*(8), 1287-1298. doi:10.1111/j.1462-  
640 5822.2012.01799.x

641 Hsu, T., Hingley-Wilson, S. M., Chen, B., Chen, M., Dai, A. Z., Morin, P. M., . . . Jacobs, W.  
642 R., Jr. (2003). The primary mechanism of attenuation of bacillus Calmette-Guerin is a  
643 loss of secreted lytic function required for invasion of lung interstitial tissue. *Proc*  
644 *Natl Acad Sci U S A*, *100*(21), 12420-12425.

645 Isberg, R. R., O'Connor, T. J., & Heidtman, M. (2009). The *Legionella pneumophila*  
646 replication vacuole: making a cosy niche inside host cells. *Nat Rev Microbiol*, *7*(1),  
647 13-24. doi:10.1038/nrmicro1967

648 Jimenez, A. J., Maiuri, P., Lafaurie-Janvore, J., Divoux, S., Piel, M., & Perez, F. (2014).  
649 ESCRT machinery is required for plasma membrane repair. *Science*, 343(6174),  
650 1247136. doi:10.1126/science.1247136

651 Kim, B. R., Kim, B. J., Kook, Y. H., & Kim, B. J. (2020). Mycobacterium abscessus infection  
652 leads to enhanced production of type 1 interferon and NLRP3 inflammasome  
653 activation in murine macrophages via mitochondrial oxidative stress. *PLoS Pathog*,  
654 16(3), e1008294. doi:10.1371/journal.ppat.1008294

655 Kimmey, J. M., Huynh, J. P., Weiss, L. A., Park, S., Kambal, A., Debnath, J., . . . Stallings, C.  
656 L. (2015). Unique role for ATG5 in neutrophil-mediated immunopathology during M.  
657 tuberculosis infection. *Nature*, 528(7583), 565-569. doi:10.1038/nature16451

658 Krakauer, T. (2019). Inflammasomes, Autophagy, and Cell Death: The Trinity of Innate Host  
659 Defense against Intracellular Bacteria. *Mediators Inflamm*, 2019, 2471215.  
660 doi:10.1155/2019/2471215

661 Kroesen, V. M., Madacki, J., Frigui, W., Sayes, F., & Brosch, R. (2019). Mycobacterial  
662 virulence: impact on immunogenicity and vaccine research. *F1000Res*, 8,  
663 doi:10.12688/f1000research.20572.1

664 Kroken, A. R., Chen, C. K., Evans, D. J., Yahr, T. L., & Fleiszig, S. M. J. (2018). The Impact  
665 of ExoS on Pseudomonas aeruginosa Internalization by Epithelial Cells Is  
666 Independent of fleQ and Correlates with Bistability of Type Three Secretion System  
667 Gene Expression. *mBio*, 9(3). doi:10.1128/mBio.00668-18

668 Kupz, A., Zedler, U., Staber, M., Perdomo, C., Dorhoi, A., Brosch, R., & Kaufmann, S. H.  
669 (2016). ESAT-6-dependent cytosolic pattern recognition drives noncognate  
670 tuberculosis control in vivo. *J Clin Invest.*, 126(6), 2109-2122. .

671 Laencina, L., Dubois, V., Le Moigne, V., Viljoen, A., Majlessi, L., Pritchard, J., . . . Girard-  
672 Misguich, F. (2018). Identification of genes required for Mycobacterium abscessus  
673 growth in vivo with a prominent role of the ESX-4 locus. *Proc Natl Acad Sci U S A*,  
674 115(5), E1002-E1011. doi: 10.1073/pnas.1713195115. Epub 1713192018 Jan  
675 1713195117.

676 Lawarée, E., & Custódio, R. (2019). Freeze! Secretion systems caught in the act. *Nat Rev*  
677 *Microbiol*, 17(2), 66. doi:10.1038/s41579-018-0145-6

678 Le Chevalier, F., Cascioferro, A., Frigui, W., Pawlik, A., Boritsch, E. C., Bottai, D., . . .  
679 Brosch, R. (2015). Revisiting the role of phospholipases C in the virulence of  
680 Mycobacterium tuberculosis. *Sci Rep.*, 5, 16918. doi:doi: 10.1038/srep16918

681 Leake, E. S., Myrvik, Q. N., & Wright, M. J. (1984). Phagosomal membranes of  
682 Mycobacterium bovis BCG-immune alveolar macrophages are resistant to disruption  
683 by Mycobacterium tuberculosis H37Rv. *Infect Immun*, 45(2), 443-446.

684 Lerner, T. R., Borel, S., Greenwood, D. J., Repnik, U., Russell, M. R., Herbst, S., . . .  
685 Gutierrez, M. G. (2017). Mycobacterium tuberculosis replicates within necrotic  
686 human macrophages. *J Cell Biol.*, 216(3), 583-594. doi: 510.1083/jcb.201603040.  
687 Epub 201602017 Feb 201603027.

688 Lerner, T. R., Queval, C. J., Fearn, A., Repnik, U., Griffiths, G., & Gutierrez, M. G. (2018).  
689 Phthiocerol dimycocerosates promote access to the cytosol and intracellular burden of  
690 Mycobacterium tuberculosis in lymphatic endothelial cells. *BMC Biol*, 16(1), 1.  
691 doi:10.1186/s12915-017-0471-6

692 Lerner, T. R., Queval, C. J., Lai, R. P., Russell, M. R., Fearn, A., Greenwood, D. J., . . .  
693 Gutierrez, M. G. (2020). Mycobacterium tuberculosis cords within lymphatic  
694 endothelial cells to evade host immunity. *JCI Insight*, 5(10).  
695 doi:10.1172/jci.insight.136937

696 López-Jiménez, A. T., Cardenal-Muñoz, E., Leuba, F., Gerstenmaier, L., Barisch, C.,  
697 Hagedorn, M., . . . Soldati, T. (2018). The ESCRT and autophagy machineries

698 cooperate to repair ESX-1-dependent damage at the Mycobacterium-containing  
699 vacuole but have opposite impact on containing the infection. *PLoS Pathog*, 14(12),  
700 e1007501. doi:10.1371/journal.ppat.1007501

701 Lugo-Villarino, G., & Neyrolles, O. (2014). Manipulation of the mononuclear phagocyte  
702 system by Mycobacterium tuberculosis. *Cold Spring Harb Perspect Med.*, 4(11),  
703 a018549. doi: 018510.011101/cshperspect.a018549.

704 Ma, Y., Keil, V., & Sun, J. (2015). Characterization of Mycobacterium tuberculosis EsxA  
705 membrane insertion: roles of N- and C-terminal flexible arms and central helix-turn-  
706 helix motif. *J Biol Chem*, 290(11), 7314-7322. doi:10.1074/jbc.M114.622076

707 Majlessi, L., & Brosch, R. (2015). Mycobacterium tuberculosis Meets the Cytosol: The Role  
708 of cGAS in Anti-mycobacterial Immunity. *Cell Host Microbe.*, 17(6), 733-735. doi:  
709 10.1016/j.chom.2015.1005.1017.

710 Manca, C., Tsenova, L., Bergtold, A., Freeman, S., Tovey, M., Musser, J. M., . . . Kaplan, G.  
711 (2001). Virulence of a Mycobacterium tuberculosis clinical isolate in mice is  
712 determined by failure to induce Th1 type immunity and is associated with induction of  
713 IFN-alpha /beta. *Proc Natl Acad Sci U S A*, 98(10), 5752-5757. .

714 Manzanillo, P. S., Shiloh, M. U., Portnoy, D. A., & Cox, J. S. (2012). Mycobacterium  
715 tuberculosis activates the DNA-dependent cytosolic surveillance pathway within  
716 macrophages. *Cell Host Microbe*, 11(5), 469-480. doi:S1931-3128(12)00125-4  
717 [pii]10.1016/j.chom.2012.03.007

718 McDonough, K. A., Kress, Y., & Bloom, B. R. (1993). Pathogenesis of tuberculosis:  
719 interaction of Mycobacterium tuberculosis with macrophages. *Infect Immun*, 61(7),  
720 2763-2773.

721 Mishra, B. B., Moura-Alves, P., Sonawane, A., Hacoheh, N., Griffiths, G., Moita, L. F., &  
722 Anes, E. (2010). Mycobacterium tuberculosis protein ESAT-6 is a potent activator of  
723 the NLRP3/ASC inflammasome. *Cell Microbiol*, 12(8), 1046-1063.

724 Mittal, E., Skowyra, M. L., Uwase, G., Tinaztepe, E., Mehra, A., Köster, S., . . . Philips, J. A.  
725 (2018). Mycobacterium tuberculosis Type VII Secretion System Effectors  
726 Differentially Impact the ESCRT Endomembrane Damage Response. *mBio*, 9(6).  
727 doi:10.1128/mBio.01765-18

728 Moreira-Teixeira, L., Mayer-Barber, K., Sher, A., & O'Garra, A. (2018). Type I interferons in  
729 tuberculosis: Foe and occasionally friend. *J Exp Med.*, 215(5), 1273-1285. doi:  
730 1210.1084/jem.20180325. Epub 20182018 Apr 20180317.

731 Moreira-Teixeira, L., Stimpson, P. J., Stavropoulos, E., Hadebe, S., Chakravarty, P., Ioannou,  
732 M., . . . O'Garra, A. (2020). Type I IFN exacerbates disease in tuberculosis-susceptible  
733 mice by inducing neutrophil-mediated lung inflammation and NETosis. *Nat Commun*,  
734 11(1), 5566. doi:10.1038/s41467-020-19412-6

735 Myrvik, Q. N., Leake, E. S., & Wright, M. J. (1984). Disruption of phagosomal membranes of  
736 normal alveolar macrophages by the H37Rv strain of Mycobacterium tuberculosis. A  
737 correlate of virulence. *Am Rev Respir Dis*, 129(2), 322-328.

738 Ngabonziza, J. C. S., Loiseau, C., Marceau, M., Jouet, A., Menardo, F., Tzfadia, O., . . .  
739 Supply, P. (2020). A sister lineage of the Mycobacterium tuberculosis complex  
740 discovered in the African Great Lakes region. *Nature Communications*, 11(1), 2917.  
741 doi:10.1038/s41467-020-16626-6

742 Novikov, A., Cardone, M., Thompson, R., Shenderov, K., Kirschman, K. D., Mayer-Barber,  
743 K. D., . . . Feng, C. G. (2011). Mycobacterium tuberculosis triggers host type I IFN  
744 signaling to regulate IL-1 $\beta$  production in human macrophages. *J Immunol*, 187(5),  
745 2540-2547. doi:10.4049/jimmunol.1100926

746 Orgeur, M., Frigui, W., Pawlik, A., Clark, S., Williams, A., Ates, L. S., . . . Brosch, R. (2021).  
747 Pathogenomic analyses of Mycobacterium microti, an ESX-1-deleted member of the

748 Mycobacterium tuberculosis complex causing disease in various hosts. *Microb*  
749 *Genom.* doi:10.1099/mgen.0.000505

750 Palmer, T., & Berks, B. C. (2012). The twin-arginine translocation (Tat) protein export  
751 pathway. *Nat Rev Microbiol.*, 10(7), 483-496. doi: 10.1038/nrmicro2814.

752 Passemar, C., Arbues, A., Malaga, W., Mercier, I., Moreau, F., Lepourry, L., . . . Astarie-  
753 Dequeker, C. (2014). Multiple deletions in the polyketide synthase gene repertoire of  
754 Mycobacterium tuberculosis reveal functional overlap of cell envelope lipids in host-  
755 pathogen interactions. *Cell Microbiol.*, 16(2), 195-213. doi: 10.1111/cmi.12214.  
756 Epub 2013 Oct 12216.

757 Paz, I., Sachse, M., Dupont, N., Mounier, J., Cederfur, C., Enninga, J., . . . Sansonetti, P.  
758 (2010). Galectin-3, a marker for vacuole lysis by invasive pathogens. *Cell Microbiol.*,  
759 12(4), 530-544. doi: 10.1111/j.1462-5822.2009.01415.x. Epub 2009 Nov 01427.

760 Peng, X., & Sun, J. (2016). Mechanism of ESAT-6 membrane interaction and its roles in  
761 pathogenesis of Mycobacterium tuberculosis. *Toxicon.*, 116:29-34.(doi),  
762 10.1016/j.toxicon.2015.1010.1003. Epub 2015 Oct 1019.

763 Picking, W. L., Nishioka, H., Hearn, P. D., Baxter, M. A., Harrington, A. T., Blocker, A., &  
764 Picking, W. D. (2005). IpaD of Shigella flexneri is independently required for  
765 regulation of Ipa protein secretion and efficient insertion of IpaB and IpaC into host  
766 membranes. *Infect Immun*, 73(3), 1432-1440. doi:10.1128/iai.73.3.1432-1440.2005

767 Pilli, M., Arko-Mensah, J., Ponpuak, M., Roberts, E., Master, S., Mandell, M. A., . . . Deretic,  
768 V. (2012). TBK-1 promotes autophagy-mediated antimicrobial defense by controlling  
769 autophagosome maturation. *Immunity*, 37(2), 223-234.  
770 doi:10.1016/j.immuni.2012.04.015

771 Pizarro-Cerdá, J., Charbit, A., Enninga, J., Lafont, F., & Cossart, P. (2016). Manipulation of  
772 host membranes by the bacterial pathogens Listeria, Francisella, Shigella and  
773 Yersinia. *Semin Cell Dev Biol*, 60, 155-167. doi:10.1016/j.semcdb.2016.07.019

774 Pym, A. S., Brodin, P., Brosch, R., Huerre, M., & Cole, S. T. (2002). Loss of RD1  
775 contributed to the attenuation of the live tuberculosis vaccines Mycobacterium bovis  
776 BCG and Mycobacterium microti. *Mol Microbiol*, 46(3), 709-717.

777 Queval, C. J., Brosch, R., & Simeone, R. (2017). The Macrophage: A Disputed Fortress in the  
778 Battle against Mycobacterium tuberculosis. *Front Microbiol.*, 8:2284.(doi),  
779 10.3389/fmicb.2017.02284. eCollection 02017.

780 Queval, C. J., Song, O. R., Carralot, J. P., Saliou, J. M., Bongiovanni, A., Deloison, G., . . .  
781 Brodin, P. (2017). Mycobacterium tuberculosis controls phagosomal acidification by  
782 targeting CISH-mediated signalling. *Cell Rep*, 20(13), 3188-3198. doi:doi:  
783 10.1016/j.celrep.2017.08.101.

784 Quigley, J., Hughitt, V. K., Velikovskiy, C. A., Mariuzza, R. A., El-Sayed, N. M., & Briken,  
785 V. (2017). The Cell Wall Lipid PDIM Contributes to Phagosomal Escape and Host  
786 Cell Exit of Mycobacterium tuberculosis. *MBio.*, 8(2).(pii), e00148-00117. doi:  
787 00110.01128/mBio.00148-00117.

788 Raynaud, C., Guilhot, C., Rauzier, J., Bordat, Y., Pelicic, V., Manganelli, R., . . . Jackson, M.  
789 (2002). Phospholipases C are involved in the virulence of Mycobacterium  
790 tuberculosis. *Mol Microbiol*, 45(1), 203-217.

791 Refai, A., Haoues, M., Othman, H., Barbouche, M. R., Moua, P., Bondon, A., . . . Essafi, M.  
792 (2015). Two distinct conformational states of Mycobacterium tuberculosis virulent  
793 factor early secreted antigenic target 6 kDa are behind the discrepancy around its  
794 biological functions. *Febs j*, 282(21), 4114-4129. doi:10.1111/febs.13408

795 Renier, S., Micheau, P., Talon, R., Hébraud, M., & Desvaux, M. (2012). Subcellular  
796 localization of extracytoplasmic proteins in monoderm bacteria: rational secretomics-

797 based strategy for genomic and proteomic analyses. *PLoS One*, 7(8), e42982.  
798 doi:10.1371/journal.pone.0042982

799 Romagnoli, A., Etna, M. P., Giacomini, E., Pardini, M., Remoli, M. E., Corazzari, M., . . .  
800 Coccia, E. M. (2012). ESX-1 dependent impairment of autophagic flux by  
801 *Mycobacterium tuberculosis* in human dendritic cells. *Autophagy*, 8(9), 1357-1370.

802 Russo, B. C., Duncan, J. K., Wiscovitch, A. L., Hachey, A. C., & Goldberg, M. B. (2019).  
803 Activation of *Shigella flexneri* type 3 secretion requires a host-induced conformational  
804 change to the translocon pore. *PLoS Pathog*, 15(11), e1007928.  
805 doi:10.1371/journal.ppat.1007928

806 Sakowski, E. T., Koster, S., Portal Celhay, C., Park, H. S., Shrestha, E., Hetzenecker, S. E., . .  
807 . Philips, J. A. (2015). Ubiquitin 1 Promotes IFN- $\gamma$ -Induced Xenophagy of  
808 *Mycobacterium tuberculosis*. *PLoS Pathog*, 11(7), e1005076.  
809 doi:10.1371/journal.ppat.1005076

810 Sayes, F., Pawlik, A., Frigui, W., Groschel, M. I., Crommelynck, S., Fayolle, C., . . . Majlessi,  
811 L. (2016). CD4+ T Cells Recognizing PE/PPE Antigens Directly or via Cross  
812 Reactivity Are Protective against Pulmonary *Mycobacterium tuberculosis* Infection.  
813 *PLoS Pathog.*, 12(7), e1005770. doi: 1005710.1001371/journal.ppat.1005770.  
814 eCollection 1002016 Jul.

815 Sayes, F., Sun, L., Di Luca, M., Simeone, R., Degaiffier, N., Fiette, L., . . . Majlessi, L.  
816 (2012). Strong immunogenicity and cross-reactivity of *Mycobacterium tuberculosis*  
817 ESX-5 Type VII Secretion- encoded PE-PPE proteins predicts vaccine potential. *Cell*  
818 *Host Microbe*, 11(4), 352-363.

819 Simeone, R., Bobard, A., Lippmann, J., Bitter, W., Majlessi, L., Brosch, R., & Enninga, J.  
820 (2012). Phagosomal Rupture by *Mycobacterium tuberculosis* Results in Toxicity and  
821 Host Cell Death. *PLoS Pathog*, 8(2), e1002507.

822 Simeone, R., Majlessi, L., Enninga, J., & Brosch, R. (2016). Perspectives on mycobacterial  
823 vacuole-to-cytosol translocation: the importance of cytosolic access. *Cell Microbiol*,  
824 18(8), 1070-1077.

825 Simeone, R., Sayes, F., Song, O., Groschel, M. I., Brodin, P., Brosch, R., & Majlessi, L.  
826 (2015). Cytosolic Access of *Mycobacterium tuberculosis*: Critical Impact of  
827 Phagosomal Acidification Control and Demonstration of Occurrence In Vivo. *PLoS*  
828 *Pathog.*, 11(2), e1004650. doi: 1004610.1001371/journal.ppat.1004650. .

829 Solans, L., Gonzalo-Asensio, J., Sala, C., Benjak, A., Uplekar, S., Rougemont, J., . . . Cole, S.  
830 T. (2014). The PhoP-Dependent ncRNA Mcr7 Modulates the TAT Secretion System  
831 in *Mycobacterium tuberculosis*. *PLoS Pathog.*, 10(5), e1004183. doi:  
832 1004110.1001371/journal.ppat.1004183. eCollection 1002014 May.

833 Sorensen, A. L., Nagai, S., Houen, G., Andersen, P., & Andersen, A. B. (1995). Purification  
834 and characterization of a low-molecular-mass T-cell antigen secreted by  
835 *Mycobacterium tuberculosis*. *Infect Immun*, 63(5), 1710-1717.

836 van der Niet, S., van Zon, M., de Punder, K., Grootemaat, A., Rutten, S., Moorlag, S., . . . van  
837 der Wel, N. N. (2020). Both adaptive immunity and IL-1R1 dependent signals  
838 improve clearance of cytosolic virulent mycobacteria *in vivo*. *bioRxiv*,  
839 2020.2009.2027.315739. doi:10.1101/2020.09.27.315739

840 van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J., . . . J., P. P.  
841 (2007). *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the  
842 cytosol in myeloid cells. *Cell*, 129, 1287-1298.

843 Wassermann, R., Gulen, M. F., Sala, C., Perin, S. G., Lou, Y., Rybniker, J., . . . Ablasser, A.  
844 (2015). *Mycobacterium tuberculosis* Differentially Activates cGAS- and  
845 Inflammasome-Dependent Intracellular Immune Responses through ESX-1. *Cell Host*

846 *Microbe.*, 17(6), 799-810. doi: 710.1016/j.chom.2015.1005.1003. Epub 2015 Jun  
847 1012.  
848 Watson, R. O., Bell, S. L., MacDuff, D. A., Kimmey, J. M., Diner, E. J., Olivas, J., . . . Cox, J.  
849 S. (2015). The Cytosolic Sensor cGAS Detects Mycobacterium tuberculosis DNA to  
850 Induce Type I Interferons and Activate Autophagy. *Cell Host Microbe.*, 17(6), 811-  
851 819. doi: 810.1016/j.chom.2015.1005.1004. Epub 2015 Jun 1012.  
852 Watson, R. O., Manzanillo, P. S., & Cox, J. S. (2012). Extracellular M. tuberculosis DNA  
853 targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell*,  
854 150(4), 803-815. doi:S0092-8674(12)00884-7 [pii]10.1016/j.cell.2012.06.040  
855 Wong, K. W., & Jacobs, W. R., Jr. (2011). Critical role for NLRP3 in necrotic death triggered  
856 by Mycobacterium tuberculosis. *Cell Microbiol*, 13(9), 1371-1384.  
857 Xiao, Y., & Cai, W. (2020). Autophagy and Bacterial Infection. *Adv Exp Med Biol*, 1207,  
858 413-423. doi:10.1007/978-981-15-4272-5\_29  
859 Zhang, L., Jiang, X., Pfau, D., Ling, Y., & Nathan, C. F. (2021). Type I interferon signaling  
860 mediates Mycobacterium tuberculosis-induced macrophage death. *J Exp Med*, 218(2).  
861 doi:10.1084/jem.20200887  
862

### 863 **Figure legends:**

864

865 **Figure 1.** Schematic view of an *Mtb*-infected phagocyte, highlighting the various signaling  
866 events induced, as described in different sections of the review. Note that the red structures  
867 drawn inside the mycobacterial cell envelope are meant to represent the mycobacterial ESX-1  
868 secretion system.

869

### 870 **Data Availability Statement**

871

872 Data sharing is not applicable to this article as no new data were created or analyzed in this  
873 study

874

### 875 **Conflict of interest statement**

876 The authors declare no conflict of interests linked to this work.

877

### 878 **Graphical Abstract text:**

879 The genome of *Mycobacterium tuberculosis*, the causative agent of human tuberculosis,  
880 harbors two loci (ESX-1 and DIM/PDIM), whose gene products are essential for the  
881 bacterium's ability to induce phagosomal rupture, when ingested by host phagocytes. The  
882 access to the host cytosol leads to important cellular signaling events and immune reactions.