Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

Review Article

The *Mycobacterium tuberculosis* capsule: a cell structure with key implications in pathogenesis

Rainer Kalscheuer[1], Ainhoa Palacios[2], Itxaso Anso[2], Javier Cifuente[2], Juan Anguita[2,3], William R. Jacobs Jr[4,5], Marcelo E. Guerin[2,3] and Rafael Prados-Rosales[2,4,6]

[1]Institute for Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; [2]CIC bioGUNE, Bizkaia Technology Park, Ed. 801A, Derio 48160, Spain; [3]IKERBASQUE, Basque Foundation for Science, Bilbao 48013, Spain; [4]Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, U.S.A.; [5]Howard Hughes Medical Institute, Albert Einstein College of Medicine, New York, NY 10461, U.S.A.; [6]Department of Preventive Medicine and Public Health and Microbiology, Autonoma University of Madrid, Madrid 28029, Spain

Correspondence: Rafael Prados-Rosales (rafael.prados@uam.es)

Bacterial capsules have evolved to be at the forefront of the cell envelope, making them an essential element of bacterial biology. Efforts to understand the *Mycobacterium tuberculosis* (Mtb) capsule began more than 60 years ago, but the relatively recent development of mycobacterial genetics combined with improved chemical and immunological tools have revealed a more refined view of capsule molecular composition. A glycogen-like α-glucan is the major constituent of the capsule, with lower amounts of arabinomannan and mannan, proteins and lipids. The major Mtb capsular components mediate interactions with phagocytes that favor bacterial survival. Vaccination approaches targeting the mycobacterial capsule have proven successful in controlling bacterial replication. Although the Mtb capsule is composed of polysaccharides of relatively low complexity, the concept of antigenic variability associated with this structure has been suggested by some studies. Understanding how Mtb shapes its envelope during its life cycle is key to developing anti-infective strategies targeting this structure at the host–pathogen interface.

Introduction

The configuration of the bacterial cell surface architecture is a product of evolution or co-evolution, including the fixation of genes involved in the synthesis of the macromolecules composing the different layers [1]. This is true for the capsule, the outermost layer of the bacterial cell, and indicates that this compartment is key for the survival strategy of bacteria. Although some exceptions exist, like the proteic poly-γ-glutamate (PGA) capsule of *Bacillus anthracis*, bacterial capsules are typically composed of high-molecular-weight acidic polysaccharides covalently linked to the cell surface [2]. Bacteria can also release exopolysaccharides (EPS) that associate with the surface and eventually encapsulate the whole cell to form slime. A clear example of this is the alginate released by *Pseudomonas aeruginosa*, representing an important virulence factor during chronic infection [3]. Nevertheless, some EPS have been shown to be closely associated with the cell surface and can also be released to the growth medium [4]. A more systematic definition of bacterial capsules has been provided by considering physicochemical parameters such as rigidity and strength of attachment to the cell [5]. As proposed by Costerton et al. [5], bacterial capsules can be classified into: (a) rigid (excludes particles); (b) flexible (does not exclude particles); (c) integral (intimately associated with the cell surface); or (d) peripheral (may be shed into the growth medium).

Bacterial capsules are usually revealed by light and/or electron microscopy techniques [2]. The fact that water accounts for up to 90% of the capsule weight is responsible for the difficulty of its visualization in a native state. The majority of the procedures to visualize bacterial capsules introduce artifacts, making it difficult to come to an accurate interpretation of such micrographs. For instance, light microscopy often requires the use of dyes that precipitate at the capsule layer to provide contrast relative to the

Received: 3 May 2019
Revised: 19 June 2019
Accepted: 20 June 2019

Version of Record published:
18 July 2019

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

whole cell and the surrounding medium [6]. Ferritin has been used to stabilize capsules, with the aim to visualize the ultrastructure by transmission electron microscopy (TEM) [7]. Common methods of fixation for TEM of whole cells can compromise the preservation of key components of the capsule, altering the overall organization [8]. To minimize the damage of conventional sample processing techniques, innovative cryogenic approaches have been developed including high-pressure freeze-substitution [9] or cryo-electron microscopy (cryo-EM) [10].

Bacteria typically organize the capsular synthetic machinery coding sequence in operons including both the genes encoding the polysaccharide synthesizing enzymes, which define the capsular serotype, and the secretion machinery that deposits the polymer at the outer surface of the cell [11]. There are three general mechanisms for bacterial capsule biosynthesis: (i) capsule synthesis mediated by the Wzx/Wzy-dependent pathway is initiated at the inner part of the membrane by the linking of the oligosaccharide unit to an undecaprenyl acceptor by glycosyltransferases (GTs), which could be transported across the membrane by a flippase (Wzx) and polymerized by the polymerase Wzy [12]; (ii) polymerization of oligosaccharides can occur in the cytoplasm before the linkage to the acceptor and the transport through the membrane conducted by an ATP-binding cassette (ABC) transporter-dependent pathway [13]; (iii) alternatively, some bacteria rely exclusively on a Wzy polymerase to synthesize the capsule, depending on export systems distinct from the canonical Wzx (synthase-dependent pathway) [14]. Fluctuations in the synthesis of capsular polysaccharides are dependent on external conditions influencing the interaction of bacteria with other bacteria or with their hosts [15]. Bacteria have developed diverse strategies to regulate capsule production that function at transcriptional level [2]. Notably, capsular polysaccharides can also be modified by the action of hydrolases removing functional groups, leading to variations in polysaccharide composition [16].

Capsular polysaccharides have been explored as targets for vaccine development due to their location at the forefront of the bacterial cell surface and their differences from human glycans. Most of the capsular polysaccharides are considered poor immunogens and need to be conjugated to proteins or carriers to elicit an appropriate immune response. The development of capsular antigenic variability by some bacteria has challenged conjugate vaccine development for many years, requiring the generation of multivalent vaccines [17]. Many functional roles have been attributed to the bacterial capsule, usually connected with colonization and persistence. The presence of the capsule in bacteria has been shown to prevent desiccation [18], promote adherence [3], provide resistance to host immunity, including complement-mediated killing [1] or down-regulate the production of pro-inflammatory cytokines [19]. A recent computational analysis seeking to identify capsular systems in prokaryote genomes revealed that half of those analyzed possess one of the canonical capsular biosynthesis systems, with some species even including more than one biosynthesis system [11].

Mycobacterium tuberculosis (Mtb) is a facultative intracellular pathogen with the ability to infect and persist in humans for decades despite the presence of a completely functioning immune system. Mtb is responsible for causing the death of almost 1.4 million people every year from tuberculosis (TB) and it is believed that one-fourth of the world population is latently infected with this bacillus [20]. One of the hallmarks of the mycobacterial survival program is the complexity of the cell envelope, which is rich in lipids and polysaccharides of unique chemical structure [21,22]. It is accepted that the intrinsically low permeability of the Mtb cell wall is partially explained by the intricate organization of its cell envelope components [23]. The mycobacterial cell envelope comprises four main layers: (i) the plasma membrane or inner membrane (IM), (ii) the peptidoglycan–arabinogalactan complex (AGP), (iii) an asymmetrical outer membrane (OM) or 'mycomembrane', that is covalently linked to AGP via the mycolic acids, and (iv) the outermost capsule [24] (Figures 1 and 2).

As previously demonstrated, the mycobacterial capsule is weakly bound to the cell wall and can be shed to the growth medium while retaining similar physicochemical properties [25]. Under laboratory conditions, the major Mtb capsule components are neutral polysaccharides, followed by proteins and lower amounts of lipids [25,26]. Therefore, the mycobacterial capsule could be defined as a peripheral capsule consisting of an EPS with the ability to remain weakly attached to the cell surface.

This review will cover the unique structure and biosynthetic pathways of the major mycobacterial capsular components and their implication in host–pathogen interactions and vaccine development.

Structure and biosynthesis of Mtb capsule

Historical perspective on the existence of a mycobacterial capsule

The quest to elucidate the mycobacterial capsule started with the discovery of Mtb and *Mycobacterium leprae* or *Mycobacterium lepraemurium* as etiologic agents of prevalent human infection diseases. Initially, this task

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

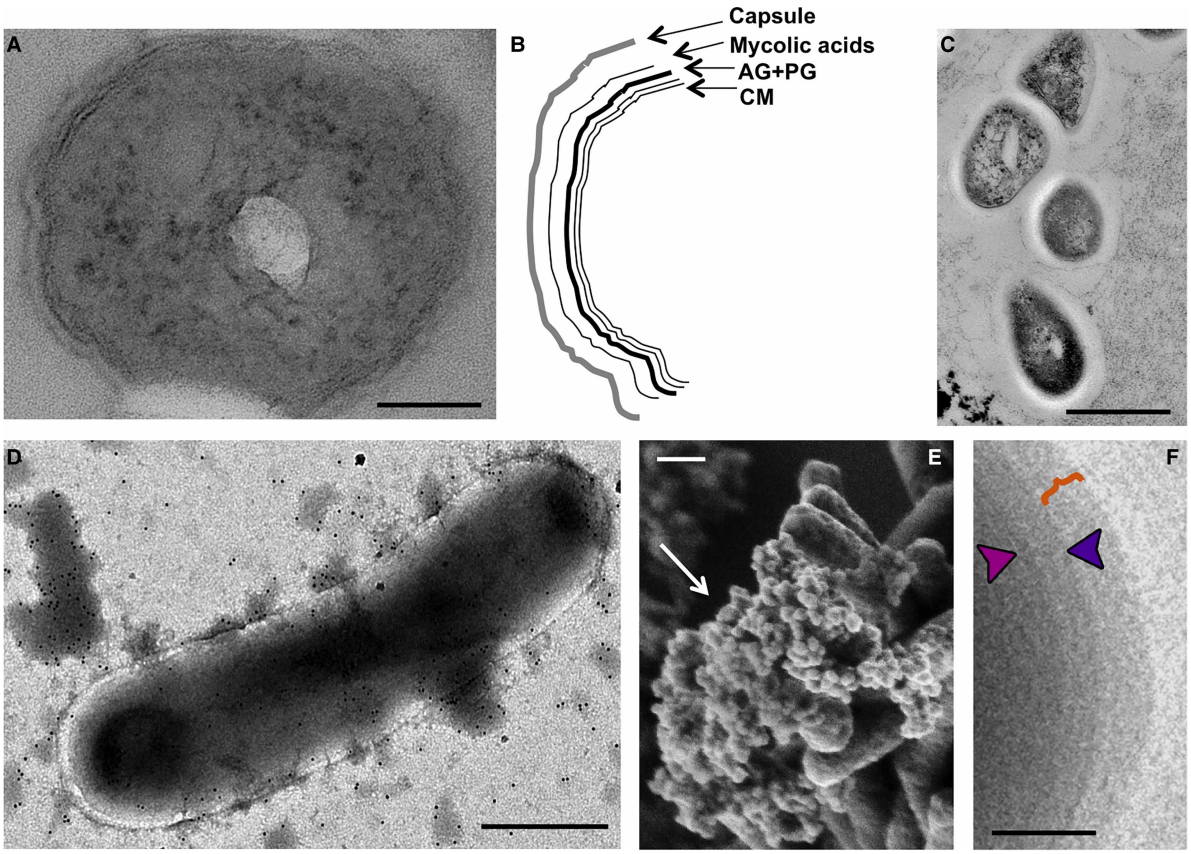

Figure 1. Visualization of Mtb capsule by electron microscopy.

(**A**) Micrograph of ultra-thin sections of Mtb grown in the absence of detergent for 24 h processed for transmission electron microscopy (TEM) [82]. Scale bar is 100 nm. (**B**) Model of the Mtb cell envelope as observed from (**A**). Note the trilaminar appearance of membrane organization. (**C**) Micrograph of ultra-thin sections of Mtb grown in the absence of detergent for 5 days processed for TEM. Note the capsule enlargement when compared with (**A**). Scale bar is 500 nm. (**D**) Electron micrograph of an Mtb cell immunostained with an anti-LAM monoclonal antibody processed for negative staining. Secondary nano-gold antibody size is 10 nm. The capsule appears as fragmented clusters of electron-dense material surrounding the bacterium. Scale bar 500 nm. (**E**) Scanning electron micrograph of Mtb grown in the absence of detergent. Arrow indicates the capsular material adhered to the bacterial clump. Scale bar 500 nm. (**F**) Cryo-electron micrograph of Mtb grown in the absence of detergent. Scale bar 100 nm. Reproduced with permission from [38].

was mostly assisted by approaches based on light and electron microscopy examining the bacillus in axenic cultures and host-infected tissues, which was associated with artifacts due to the techniques available at that time [9,24,27]. In those early studies, the presence of an 'extracellular material' or 'amorphous matrix' surrounding individual bacilli was reported [28,29]. In addition, groups of bacilli were found within a limiting membrane of unknown origin in host-infected cells [29]. Interestingly, this material was referred to as an 'electron transparent zone' in electron micrographs [30]. The nature and significance of this 'extracellular material' remained unknown and a matter of debate [27–29]. Ultra-thin sections of spleens from mice infected with *M. lepraemurium* clearly demonstrated the existence of a 'capsule space' separating a 'capsule enclosing membrane' and the cell wall of the bacillus [24,31,32]. This 'capsule space' was interpreted as a capsule of mycobacterial origin and the 'capsule enclosing membrane' as the phagocytic membrane of an infected host cell [24,31,33–36]. Subsequent studies using light microscopy and dyes further confirmed the presence of a capsule as an 'unstainable halo' surrounding several pathogenic mycobacteria, including Mtb and *Mycobacterium bovis* BCG [33]. The improvements in the preservation of cellular morphology through the embedding by freeze-substitution also provided additional evidence of the existence of this mycobacterial compartment [34,37]. It is worth mentioning that the visualization of the mycobacterial capsule greatly depends on growth conditions, since the

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

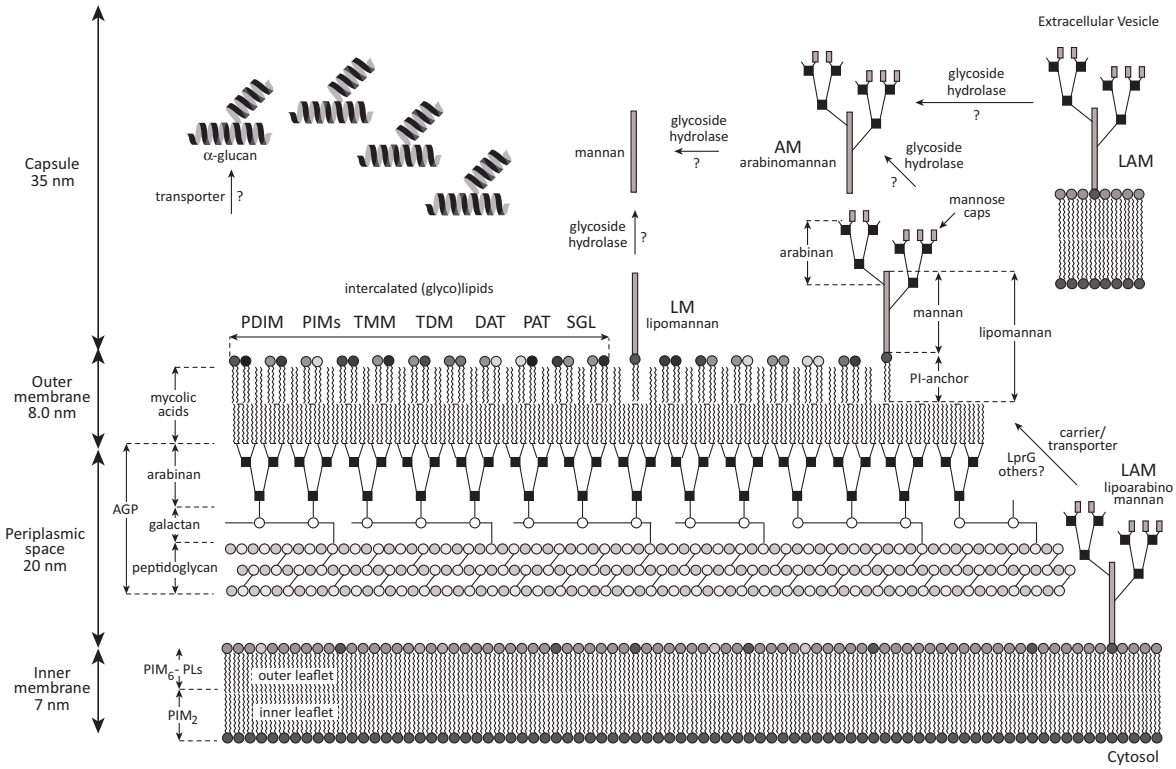

Figure 2. The Mtb cell envelope.

The cell envelope of Mtb comprises four main layers: (i) the plasma membrane or inner membrane, (ii) the peptidoglycan–arabinogalactan complex (AGP), (iii) an assymetrical outer membrane or 'mycomembrane', that is covalently linked to AGP through the mycolic acids, and (iv) the external capsule [22]. The inner leaflet of the outer membrane is composed of long chain (C60–C90) fatty acids, with the outer leaflet mostly consisting of a variety of non-covalently attached (glyco)lipids and lipoglycans, including trehalose mono- (TMM) and dimycolates (TDM), sulfoglycolipids (SGL), phosphatidylinositol mannosides (PIMs), lipomannan (LM) and lipoarabinomannan (LAM), and lipoproteins/proteins some of which are glycosylated [63,64,159]. The structural organization of this matrix is responsible for the low permeability of the Mtb cell envelope [23,24]. LAM is presumed to be non-covalently associated with the cell membrane, although it has also been found in the mycomembrane. Different studies indicate that the lipoprotein LprG could be assisting the transfer of membrane-associated LAM to the mycomembrane [66,67]. The capsule is mostly made of neutral polysaccharides including a major glycogen-like α-glucan and lower amounts of arabinomannan (AM) and mannan. Both AM and mannan are structurally and chemically similar to the cell-wall-associated LAM and LM. We hypothesize that the action of a missing enzyme could be responsible for the conversion of LAM and LM into AM and mannan, respectively. This enzyme could function at the level of the mycomembrane or the extracellular vesicles, which were shown to be carriers of LAM [68].

mycobacterial capsule is only weakly bound to the cell wall and it is easily detached by agitation or addition of detergent [38]. More recent ultrastructural studies utilizing Cryo-EM aimed to visualize the mycobacterial capsule in a close-to-native state and demonstrated its existence not only in Mtb but also in the nonpathogenic strain *Mycobacterium smegmatis* [38].

Chemical composition and biosynthesis of the Mtb capsule

The fact that early studies on mycobacterial capsule were performed in two host-grown species, *M. leprae* and *M. lepraemurium,* and the fact that both species are known to release large amounts of specific lipids, such as glycopeptidolipid (GPL), suggested that the capsule was an accumulation of those lipids within the phagosomal membrane. However, this was later ruled out when the presence of capsule could be confirmed in other mycobacterial species growing in axenic cultures, which exhibit an impaired or highly reduced ability to release these

lipids [39]. Importantly, the use of ruthenium red and peroxidase-conjugated concanavalin A in these studies suggested the polysaccharide nature of this compartment [39,40]. More recent work, pioneered primarily by Daffe's group, demonstrated that the mycobacterial capsule consists of neutral polysaccharides, proteins and lower amounts of lipids [25,26]. It was determined that polysaccharides are the major capsular components in slow-growing mycobacterial species while proteins seem to be the main constituents in fast-growers such as *M. smegmatis* [25,26,41].

Capsular polysaccharides

Three main capsular polysaccharides were identified in Mtb's capsule: (i) α-D-glucan is a branched high-molecular-weight homopolysaccharide composed of α-D-glucose (α-D-Glc) residues connected by α-1,4-linkages to form a linear core, partially substituted at position 6 with additional α-D-Glc residues [42] and an apparent molecular mass of 100 kDa [26,43,44] (Figure 3A); (ii) D-arabino-D-mannan (AM) is a hetero-polysaccharide that exhibits an apparent molecular mass of 13 kDa, comprising D-mannan linked to an D-arabinose-branched polysaccharide containing α-1,3, α-1,5 and β-1,2 glycosidic linkages [25,26,43] (Figure 4); (iii) D-mannan, is a homopolysaccharide that exhibits an apparent molecular mass of 4 kDa, composed of α-D-mannose (α-D-Man) residues connected by α-1,6-mannosidic linkages, substituted at some of the two positions with an α-D-Man residue [26,43] (Figure 4).

α-D-glucans are found in bacteria and eukaryotes as discrete, mostly spherical, intracellular cytoplasmic particles of different size serving as a carbon storage compound, commonly referred to as glycogen [45]. The classical biosynthetic pathway of bacterial α-glucan involves the action of three enzymes. First, ADP-glucose pyrophosphorylase (AGPase, *glgC*) converts ATP and glucose-1-phosphate (G1P) into the activated nucleotide sugar donor ADP-glucose. Importantly, AGPase senses cell metabolic energy reporters to exert an allosteric

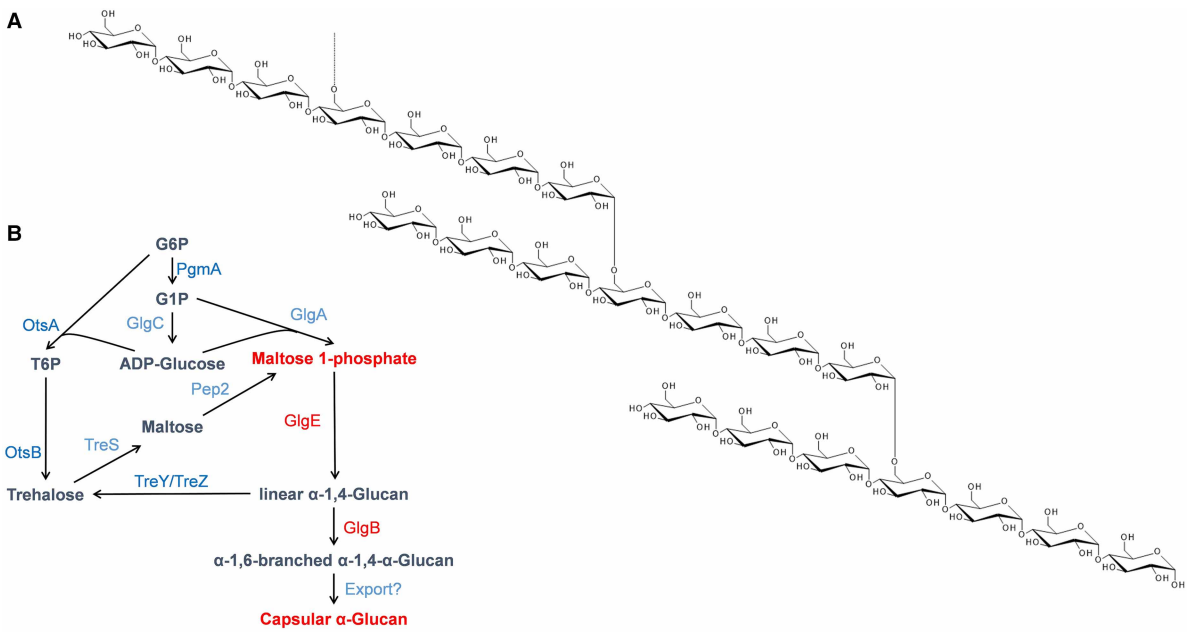

Figure 3. Chemical structure and biosynthetic pathway of α-glucan from Mtb.

(**A**) Structure of intracellular (=glycogen-like) and extracellular (=capsular) α-glucan from Mtb. The polymer consists of linear α-1,4-glucan chains comprising ca. seven glucose units, each bearing mostly only one α-1,6-branch. This results in a polymer that exhibits a significantly lower degree of branching, and hence a less arboreal structure, compared with glycogen from other bacteria or mammals. (**B**) Pathway for biosynthesis of glycogen-like and capsular α-glucan in Mtb (adopted from [54]). The two essential enzymes GlgE and GlgB co-operate in an iterative process to produce a structurally distinct α-glucan as depicted in **A** using maltose 1-phosphate as substrate. The polymer is produced intracellularly and then partially secreted to build the capsule. The substrate maltose 1-phosphate is synthesized on two alternative routes which are interconnected by the shared use of ADP-glucose and allows rechanneling of ADP-glucose and buffering of total maltose 1-phosphate formation.

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

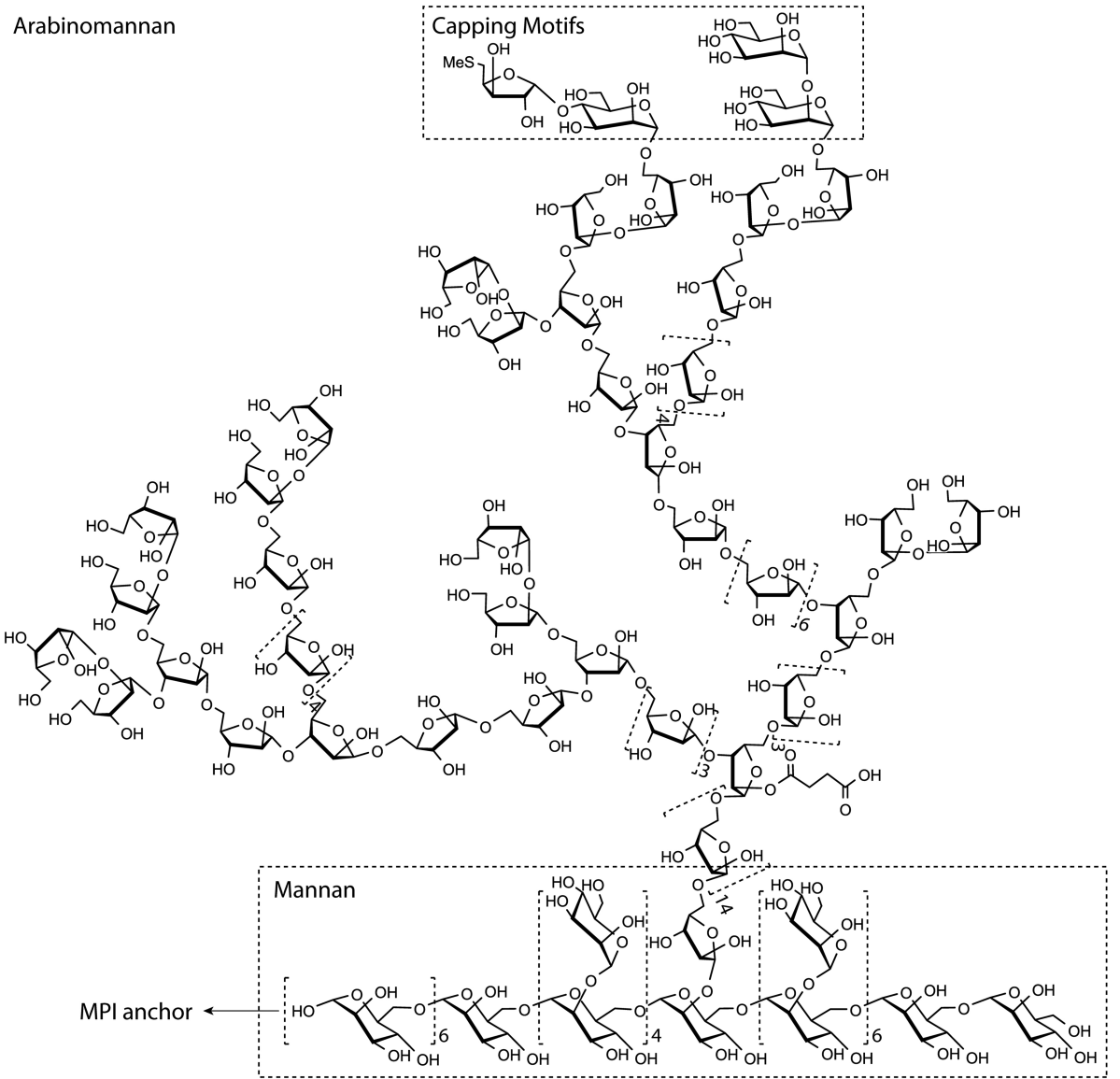

Figure 4. Chemical structure of Mtb mannan (M) and arabinomannan (AM).
Details on the biosynthetic pathway can be found in [21,22].

control of the reaction [46]. The second step is carried out by the glycogen synthase (GS, *glgA*), a GT that employs ADP-glucose to generate linear α-1,4-linked glucose chains [47]. Finally, the branching enzyme (BE, *glgB*), a glycoside hydrolase (GH), promotes the formation of α-1,6-linked branches in the linear polymer [48]. α-Glucan degradation involves the action of two enzymes: the glycogen phosphorylase (GP, *glgP*), which functions as a depolymerizing enzyme, and the debranching enzyme (DBE, *glgX*) that catalyzes the removal of α-1,6-linked branches [46,47]. Interestingly, several bacteria organize all these enzymes in a single operon, such as *glgBXCAP* in *Escherichia coli* [49]. The uniqueness of Mycobacterial glycogen synthesis is reflected by the fact that genes encoding the biosynthetic enzymes are not structured in a single operon and, besides its intracellular localization, mycobacterial α-D-glucan is also deposited extracellularly as a capsular component [42].

In Mtb, α-1,6-branched α-1,4-glucans are produced by iterative cooperation of two essential enzymes, the maltosyltransferase GlgE and the branching enzyme GlgB [50]. The maltosyltransferase GlgE is the only enzyme responsible for producing linear α-1,4-D-glucan chains in mycobacteria as mycobacterial GlgA has a different function, to be discussed below. GlgE uses the phosphosugar maltose 1-phosphate as the activated

donor substrate, which is unusual since most known GTs rely on nucleotide-sugars as donor substrates [51,52]. As soon as GlgE has formed a linear chain of ∼16 glucosyl residues, GlgB introduces an α-1,6 branch of ∼7–8 glucosyl residues in length employing a strictly intrachain transfer mechanism. GlgE then preferentially extends the newly formed branch until it is long enough to undergo branching again by GlgB. Only occasionally, GlgE also extends previous branches so that they might become long enough to allow a second branch to be introduced by GlgB. Therefore, each branched chain mostly carries just one further branch. These specificities of GlgE and GlgB promote an iterative process that results in a glucan polymer which differs from glycogens produced by other bacteria by exhibiting a significantly lower degree of branching, resulting in a less pronounced arboreal structure compared with glycogen from other bacteria and from mammals [50] (Figure 3A). It is unknown whether these distinct structural features of mycobacterial glucan are important for export of the polymer across the different layers of the cell wall or for interaction with the host's immune response. However, a monoclonal murine antibody raised against Mtb capsular glucan also recognizes mammalian glycogen, indicating that glucans from different sources are antigenically similar despite structural differences [53]. The substrate of GlgE, maltose 1-phosphate, is provided by two alternative, intertwined routes. In the first pathway, the disaccharide trehalose (α-1,1-diglucose) is isomerized to maltose (α-1,4-diglucose) by trehalose synthase TreS, followed by ATP-dependent phosphorylation of maltose to maltose 1-phosphate catalyzed by the maltokinase Pep2 [51]. The second route is a variant of the classical GlgC–GlgA pathway, which is used for the direct formation of glycogen in other bacteria but has a different specificity and function in Mtb. Mycobacterial GlgA is not a glycogen synthase, but produces maltose 1-phoshate by transferring glucose from ADP-glucose to glucose 1-phosphate. Thus, GlgA has been suggested to be renamed GlgM to reflect this different substrate specificity and enzymatic activity (to reflect this peculiarity, we have named GlgA as GlgM through the text) [54]. ADP-glucose, in turn, is synthesized from glucose 1-phosphate by the ADP-glucose pyrophosphorylase GlgC as in other bacteria. ADP-glucose is also used as a precursor for trehalose biosynthesis by the trehalose 6-phosphate synthase OtsA, which transfers glucose from ADP-glucose to D-glucose-6-phosphate. The shared use of the intermediate ADP-glucose by GlgM (GlgA) and OtsA links the TreS–Pep2 and GlgC–GlgA pathways and allows for flexibility in generating the maltose 1-phosphate building block for GlgE-mediated α-glucan production in mycobacteria [54] (Figure 3B). Of note, biosynthetic genes of this pathway are distributed into three different clusters. A first gene cluster includes *glgC* and *glgM* (*glgA*) together with *gpgS,* the latter involved in α-1,4-methylglucose lipopolysaccharide biosynthesis [55,56]. A second cluster of genes includes *glgB, glgP* and *glgE* [57]. Finally, *treX* (Mtb homolog of *glgX*) is located in a third cluster with *treY* and *treZ* and is implicated in trehalose synthesis from α-1,4-glucan.

The structural identity of the intra- and extracellular polymers implies that capsular α-glucans have an intracellular biosynthetic origin. This is supported by the fact that disturbed activity of the sole polymerase GlgE results in intracellular accumulation of its substrate maltose 1-phosphate [51]. Furthermore, the incubation of purified GlgE and GlgB proteins with maltose 1-phosphate *in vitro* is sufficient to yield an α-glucan polymer that is structurally identical with glycogen and capsular glucans isolated from Mtb cells [50]. This Mtb α-glucan displays a lower degree of branching resulting in a less pronounced arboreal structure compared with glycogen from other bacteria and eukaryotes [44] (Figure 3A). Thus, a dedicated transporter for α-glucan secretion likely exists in Mtb, which has not yet been identified. The recent full elucidation of the complex biochemical network underlying α-glucan production in mycobacteria has allowed the creation of an Mtb double mutant that is defective in both routes of maltose 1-phosphate biosynthesis. This double mutant lacked the glucan capsule and showed reduced virulence in mice, suggesting an important role for capsule in virulence. However, this mutant is also defective in intracellular glycogen production, making it difficult to determine the specific contribution of capsular glucans to virulence of Mtb [54].

The structure of capsular AM and mannan appear to be identical with the carbohydrate moieties of two of the major mycobacterial cell envelope-associated lipopolysaccharides, lipoarabinomannan (LAM) and lipomannan (LM), respectively [25,26,43]. Consequently, the biosynthetic machinery of these capsular polysaccharides shares most of the genes with that of LAM and LM [22] (Figure 4). Importantly, LAM is modified as its most terminal part by mannosyl caps (Figures 2 and 4) and, technically, this molecule should be named as ManLAM. In fact, this modification is exclusive of slow-growing mycobacteria as fast-growing mycobacteria such as *M. smegmatis* produces phosphatidyl-myo-inositol capped lipoarabinomannan (PILAM) [58]. There is another variation of LAM consisting of a polysaccharide devoided of any mannosyl cap, which is named AraLAM. It has been also reported that LAM can also be modified with a 5-methylthio-d-xylofuranose (MTX) [59,60]. Although this substitution is very rare (one MTX per LAM molecule), it seems to have implications in

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

the improvement of LAM immunodetection assays by the inclusion of MTX-binding monoclonal antibodies (mAbs) [61].

In order for LAM and LM to be transformed into capsular AM and mannan, an additional and unknown enzymatic step involved in the removal of the lipid tail should exist [62]. Several reports indicate that the exposure of LAM and LM is restricted to either the periplasmic space [63,64] or the OM [65]. How LAM reaches this later compartment is currently unknown. Interestingly, two independent studies proposed a role for the lipoprotein LprG in assisting the transfer of LAM from the cell membrane to the cell surface [66,67] (Figure 2). This scenario positions the missing enzyme in two different locations, suggesting that AM and mannan could be generated either at the membrane level or at the mycomembrane. There is a complementary hypothesis regarding the synthesis of these two capsular PS, which is related to the ability of Mtb to release extracellular vesicles (EVs) [68]. In a manner similar to other bacteria, Mtb and many other mycobacterial species produce EVs. Mycobacterial EVs from pathogenic strains are enriched in lipoproteins, which are well-known immunomodulatory molecules, and induce a TLR-2-dependent inflammatory response *in vitro* and *in vivo*. Lipidomic analysis indicated that these structures are originated at the cell membrane and vesiculation was observed while Mtb was confined within the phagosomes of bone marrow macrophages (BMMs). This suggests that EV production may provide an alternative mechanism for transport of immunomodulatory compounds, leading to the distribution of these compounds to various compartments within infected host cells or to exosomes. Although the mechanism by which such vesicles are exported across the mycobacterial cell wall is not known, similar vesicles have been described in other microbes with dense cell walls such as Gram-positive bacteria and fungi [69]. In addition to lipoproteins, EVs are loaded with LAM. Considering the surface exposure of LAM in these EVs, it is possible to hypothesize that the action of the missing enzyme could be happening at the vesicle level (Figure 2). As mentioned above, the potential exposure of LAM could also be influenced by the use of detergent in the growth medium. Unencapsulated mycobacteria would expose cell wall-associated LAM and LM, contrary to what would occur in the absence of detergent, where most probably capsular AM and mannan could be present at the outermost surface of the cell [26,41]. The route by which capsular AM and mannan are produced and deposited has key implications in the study of the initial interaction between Mtb and cells of the innate immune system.

In vitro studies have demonstrated time-dependent changes of α-glucan, AM and mannosylated lipoglycans such as LAM [53,70,71]. Under laboratory conditions α-D-glucan appears to be the major capsular component [53]. However, it is unknown, whether this is true under different growth conditions, including *in vivo* growth. Taking into consideration the proposed general mechanisms for bacterial capsule biosynthesis, it is conceivable that AM and α-glucan could have different mechanisms of synthesis and transport. AM synthesis could be performed via a type (i) Wzx/Wzy-dependent pathway, which initiates at the inner part of the membrane, where the precursor glycolipid phosphatidyl inositol mannoside 4 (PIM_4) is believed to be flipped by an unidentified flippase such that its sugar moiety is extended at the other side of the membrane by several integral glycosyltranferases to generate LAM. Subsequently, LAM would be processed to form AM. α-glucan synthesis could fit into a type (iii) pathway where polymerization of oligosaccharides occurs in the cytoplasm prior to the export of the mature polysaccharide. Nevertheless, more studies are needed to gain insight into the dynamics of Mtb capsule synthesis and the relative configuration of its main components.

Capsular proteins and lipids

It is assumed that the association of proteins and lipids in the mycobacterial capsule occurs during their secretion or release. This is because the composition of the culture filtrate extract and that of capsular extract is very similar [26]. Early attempts to characterize the molecular composition of the capsule included the proteomic and lipidomic analysis of the material mechanically extracted by glass beads from Mtb cells grown in the absence of detergent. Although this analysis was restricted to a comparison of SDS–PAGE protein profiles between culture filtrate, cytosol and capsule extracts, some proteins were enriched in the latter compartment, including the lipoproteins LpqH and PstS1. Similarly, some proteins from the Ag85 complex were found to be associated with the Mtb capsule [26]. The same group reported in a different study the association of proteins such as BlaC, Ald, Adh, KatG, GlnA1, PncA and SodA with the capsule extract of Mtb through the measurement of their corresponding enzymatic activities [72]. More recently, as part of a study aiming to visualize the mycobacterial capsule in a native state, high-throughput proteomics was performed on capsule extracts of different mycobacteria such as Mtb, *Mycobacterium marinum* or *M. smegmatis* [38]. Of note, ESAT-6 secretion system-1 (ESX-1) associated proteins were found in high amounts only in a detergent-extracted capsule of

M. marinum and were absent from extracts from Mtb and *M. smegmatis*. The ESX-1 secretion system is essential for Mtb pathogenesis and it seems to be dedicated to the delivery of very active proteins whose primary function is to alter some critical steps of the innate immune response [73,74]. The presence of ESX-1-associated proteins in the capsule of *M. marinum* would suggest that the capsule itself is a reservoir of virulence-related molecules.

Experiments are needed to determine whether this variability in capsular protein composition among mycobacterial strains is due to differential sensitivity to detergent extraction, which could be attributed to differences in the avidity with which proteins are associated with the capsule.

The composition of capsular lipids of Mtb and other mycobacterial species was initially determined by the progressive removal of the cell envelope using different mechanical and chemical methods and analysis by thin-layer chromatography [41]. This study showed that the Mtb lipid species most exposed to the extracellular space were phosphatidyl-*myo*-inositol mannosides (PIMs), diacyl trehaloses (DATs), phthiocerol dimycocerosates (PDIMs) and phosphatidylethanolamine (PE). Similar to the proteomic study mentioned above, species-specific differences in the lipid composition of the capsule were found among different mycobacterial strains. Although some of these differences could be attributed to the presence of some species-specific lipids, additional studies are needed to ascertain the degree of association of these lipids with the outermost compartment. Notably, a high-throughput lipidomic analysis of the mycobacterial capsule using the current technologic capabilities, including liquid chromatography coupled to mass spectrometry (LC–MS), would help define the molecular composition of the mycobacterial capsular lipids.

The mycobacterial capsule in the context of host–pathogen interaction

Many intracellular pathogens have developed strategies to invade phagocytic cells that enable intracellular survival and persistence. Part of Mtb's survival strategy depends on the initial mode of interaction with host cells, where the outermost compartment of the mycobacterial cell envelope plays a key role. Early studies clearly demonstrated that the capacity of Mtb to restrict phagolysosomal fusion, a cellular function which is essential to destroy intracellular pathogens, is lost when the bacterium is marked for ingestion (opsonized) prior to the entry to macrophages [75]. Conversely, the nonopsonic or direct internalization of Mtb by phagocytes favors the intracellular survival of the bacillus [76]. According to this, the mycobacterium infection is a dynamic process and results in a changing environment including different type of lung cells with the capacity to internalize Mtb or modulate the function of other immune cells. The repertoire of lung cells with the capacity to internalize Mtb in the alveolar space includes professional phagocytes like alveolar macrophages and submucosal and interstitial dendritic cells (DCs) and also nonphagocytic cells like epithelial cells [77]. Mtb can enter phagocytes nonopsonically or after being opsonized through a variety of phagocytic receptors including mannose receptor (MR), complement receptors (CR, CR1, CR3 and CR4), surfactin protein A (Sp-A) receptor, the DC-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), Fc receptors (FcRs), scavenger receptors, CD14 and CD44 (Table 1) [78]. Alternatively, Mtb can also stimulate lung cells by engaging nonphagocytic receptors inducing distinct immunomodulatory programs.

The biological properties of encapsulated and unencapsulated mycobacteria may diverge extensively and the array of possible ligands on display to readily interact with the also varied repertoire of host receptors may be different. This is particularly important when assessing the role of receptors in mycobacterial uptake by phagocytes; therefore, conclusions derived from studies including unencapsulated mycobacteria should be taken with caution. Supporting this notion, several studies have demonstrated that manipulation of mycobacterial cultures in the context of vaccination studies influences the outcome of the interaction with the host [79,80]. Inconsistencies between different studies addressing the role of specific Mtb cell envelope ligands in the outcome of the interaction with phagocytic cells might have several different explanations: (1) the growth conditions of Mtb can influence the composition of its cell envelope. For instance, growing the infecting strain with detergent renders the bacterium unencapsulated, exposing cell wall lipids or lipoglycans [41,81]; (2) the chosen method to disrupt mycobacterial aggregates will have an effect on the preservation of the mycobacterial capsule, with the use of glass beads or sonication distorting the mycobacterial cell envelope to a greater extent [41,82]; (3) even in the absence of detergent the Mtb's cell surface is most probably not homogeneous (Figure 1) and this creates multiple scenarios of interaction with phagocytic cells via different receptors; and (4) little attention was given to the purity of isolated capsular or cell wall Mtb ligands as contaminants in these preparations have been reported [83]. From the host's perspective, the availability of functional receptors on

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

Table 1 Phagocytic receptors that mediate the opsonic or nonopsonic binding of Mtb

Receptor	Cell type	Manipulation of Mtb*	Ligand	Type of binding	Refs
Mannose receptor (MR)	Human macrophages	GB	LAM	Nonopsonic	[94,95]
	Human macrophages	GB	Mannan	Nonopsonic	[94]
	Human macrophages	GB	PIM5, PIM6	Nonopsonic	[123]
Complement receptor 1 (CR1)	Blood monocytes	D	C3b, C4b	Opsonic	[91]
Complement receptor 3 (CR3)	CHO-CR3, BMDMs, Alveolar macrophages	S	α-glucan	Nonopsonic	[86–89]
	CHO-CR3, Blood monocytes	GB, D	iC3b	Opsonic	[87,91]
	Monocytes-derived macrophages	GB	PIM2	Nonopsonic	[122]
Complement receptor 4 (CR4)	Alveolar macrophages	D	iC3b	Opsonic	[91]
	Human macrophages	D	SP-A	Opsonic	[100]
SPA-R	Human macrophages	D	SP-A	Opsonic	[102]
?	Human macrophages	GB	SP-D	Opsonic	[105]
CD43	BMDMs	D	GroEL	Nonopsonic	[116]
DC-SIGN	Human dendritic cells	GB	LAM, Mannan	Nonopsonic	[113]
	COS-1-DC-SIGN	GB	LM, PIM5, PIM6	Nonopsonic	[123]
Fc receptor	BMDMs	D	IgGs	Opsonic	[110]
	Human macrophages	GB	IgGs	Opsonic	[111]
Scavenger receptors	Human PBMCs	D	Unknown	Nonopsonic	[93]

*D, detergent; GB, glass beads; S, syringe; Sc, sonication.

the surface of resident and recruited lung cells as well as the abundance of opsonins (markers for ingestion) at the site of interaction should be taken into consideration since they can dictate the mode of entry of Mtb.

Role of the capsule in the binding and uptake of Mtb by lung cells

Several studies have demonstrated that the outermost capsular layer of the mycobacterial cell envelope modulates the uptake of Mtb by lung cells. The antiphagocytic capacity of the Mtb's capsule during the interaction with different types of primary macrophages and THP-1 macrophage-like cells in the absence of opsonins has been demonstrated [82]. In a more recent study, it was shown that the presence of the mycobacterial capsule enhances the uptake of serum-opsonized encapsulated Mtb and induces an enhanced pro-inflammatory response by human monocyte-derived macrophages (HMDM) relative to unencapsulated Mtb [38]. These and other examples, including the modulation of macrophage functions such as binding and phagocytic capacity for Mtb by capsular components, are discussed below. It is worth to highlight here the recent development of a resource consisting of a glycan array including a set of representative chemically synthesized Mtb-derived sugars, some of them capsule-related, which was used to study their specific interaction with a set of soluble receptors from innate immune cells [84]. Results from this study validated previously established interactions, and reported overlapping interactions for several glycans. Importantly, no interaction between any receptor and furanoses was found, even though this residue is recognized by Mtb-specific antibodies (Abs) [85].

Capsular polysaccharide-mediated binding and uptake by macrophages

Regarding the role of capsular mycobacterial polysaccharides as ligands for phagocytic receptors, both CR3 and MR have been extensively studied. Of particular interest is the finding that capsular α-glucan is the mediator

for the direct binding of Mtb to the CR3 lectin site of this integrin as shown in experiments using Chinese hamster ovary (CHO) cells expressing CR3 at the membrane [86,87]. Importantly, nonpathogenic strains of mycobacteria or unencapsulated Mtb required previous opsonization for their internalization via CR3 and binding was restricted to the CR3bi site [87]. It is well documented that many intracellular pathogens chose to gain access to phagocytic cells via direct binding to CR3 to avoid their clearance, indicating that the α-glucan-mediated binding and entry to phagocytic cells via CR3 may represent a favorable route of entry for Mtb. These studies show clear evidence for the key role of the capsular glucan during the initial interaction with macrophages, yet the use of CHO cells solely expressing a single receptor does not offer the possibility of interrogating such interactions in the context of the multiple receptors. Although these studies were confirmed by similar experiments using bone marrow-derived macrophages (BMDMs) [88] or alveolar and resident macrophages [89] from CR3−/− mice, they showed no role for CR3 in the intracellular fate of Mtb after infection. In addition, they showed no differences in mycobacterial uptake at higher multiplicities of infection or when prolonging the initial interaction between Mtb and macrophages, suggesting that multiple receptors can be used to gain entry to phagocytes in the absence of CR3 [88,90]. In a similar study, it was shown that Mtb, when opsonized with fresh serum, was preferentially ingested via CR4 by human alveolar macrophages, whereas CR1 and CR3 played a major role in blood monocytes [91] (Table 1). Although these studies were performed with bacteria grown in the presence of detergent, it clearly shows that the macrophage environment may determine the mode of interaction with Mtb. *In vivo* experiments using up to three mouse strains with different susceptibility to Mtb showed no defects in controlling Mtb infection of CR3−/− mice relative to WT mice [88]. Of note, however, no details about whether the infecting Mtb strains were encapsulated were provided in these studies and, more importantly, the development of a potential compensatory effect upon CR3 deletion was never investigated. Nevertheless, considering that in the alveolar space of a quiescent lung active complement components are scarce, direct binding of Mtb to lung macrophages may represent an important path of entry [92]. Further investigation is required to clarify the role of CR3, considering the questioned availability and functionality of CR3 on resident lung macrophages during the initial and subsequent stages of infection [91,93]. Although mouse studies have shed light on the molecular interaction between Mtb and phagocytic cells, these studies should be taken with caution since differences in the expression, activity and availability of complement receptors between mouse and human macrophages exist.

Mtb can also be phagocytosed by macrophages either opsonically or nonopsonically via the MR. Typically, this C-type lectin receptor recognizes mannose, fucose and *N*-acetylglucosamine exposed sugars and is prevalently expressed on mature non-activated phagocytes. The molecular interaction of Mtb mannosylated ligands with phagocytes has been a topic of intense investigation (reviewed in [73]). Several studies demonstrated the direct engagement of unencapsulated Mtb with the MR in human macrophages via LAM [94], leading to the subsequent blocking of macrophage phagosome–lysosome function [95]. Importantly, the downstream signaling events leading to the limiting of phagosome–lysosome fusion after the engagement of LAM with human MR have recently been identified [96]. Similarly, it has been shown that engagement of Mtb to MR via LAM triggers an anti-inflammatory program including suppression of IL-12 [97] and reactive oxygen species production [98]. As previously mentioned for CR3, several facts should be considered in evaluating the importance of MR during the interaction of phagocytes with Mtb: (i) the expression of MR is high in resident differentiated macrophages in a quiescent lung [99] and (ii) activation of macrophages leads to the down-regulation of MR [100]. This suggests that MR might have key roles during the initial stages of the infection process and a minor role after macrophage activation. Considering that LAM is not physically located at the outermost space of the Mtb's cell envelope [26,41], capsular polysaccharides including AM or mannan could be natural ligands for MR. This hypothesis, however, awaits further experimental evaluation.

Another set of lung components with capacity to bind the surface of Mtb are the collectins, including the surfactant binding proteins A and D (SP-A and SP-D) and the mannose-binding lectin (MBL) [101]. The importance of collectins relies on their ability to not only mediate the uptake of pathogens but also to influence the inflammatory status of the lung. SP-A mediates the uptake of mycobacteria by alveolar mononuclear phagocytes [102] and HMDM or alveolar macrophages [103] and shows similar binding selectivity to MR, suggesting that similar ligands might be targeted by these molecules. Indeed, both terminal mannose residues and the lipidic anchor of LAM were shown to be required for binding to SP-A [104]. It was speculated that the lipid portion of LAM was required to create a supramolecular LAM structure that could favor the interaction with SP-A. This scenario opens the discussion about the potential interaction of SP-A with similar ligands such as capsular AM or mannan as the way these polysaccharides are organized within the capsule is currently unknown.

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

Similar to SP-A, the binding of SP-D to Mtb LAM has also been demonstrated [105]. Moreover, SP-D could not directly bind *M. smegmatis* cells and only bind poorly to PILAM from this species, suggesting a preference of SP-D for virulent mycobacteria. However, it cannot be ruled out that aspects related to the spatial orientation of LAM as well as differential exposure of these molecules on the surface of avirulent mycobacteria could account for these differences. Contrary to SP-A, SP-D seems to promote mycobacterial aggregation and reduction in phagocytosis by human macrophages, possibly by blocking the access of Mtb to MR, which suggests a protective role of SP-D against Mtb.

MBL is another collectin, predominantly found in serum [106] and has been shown to enhance mycobacterial phagocytosis by human neutrophils [107] or endothelial cells [108] through binding to LAM or PIMs, respectively. The capacity of MBL to activate complement could be used by Mtb to gain access to macrophages to its own benefit. Consequently, high levels of MBL could promote bacterial phagocytosis and establishment of the infection. Several reports support this hypothesis as high levels of MBL have been found in sera from TB patients [109].

Two independent reports have shown the role of FcR-mediated internalization and growth inhibition of Mtb by macrophages via immunoglobulins G [110,111]. Interestingly, in one report, the association of human Abs to capsular AM with an enhanced ability of human macrophages to uptake Mtb and reduce its intracellular growth was established [111]. In agreement with this, the induction of AM-specific Abs via vaccination could contribute to an enhancement in the control of bacterial dissemination in Mtb-infected mice [112].

Capsular polysaccharide-mediated binding and uptake by dendritic cells

Other professional phagocytes with capacity to ingest Mtb at the site of infection include lung submucosal and interstitial DCs. These cell populations express CRs and MR like macrophages, but it appears that the receptor playing the major role in the direct binding of Mtb to DC is DC-SIGN, and Mtb ligands mediating this binding have been identified as LAM and α-Glc [113,114]. Both lipid and mannosyl caps of LAM seem to be required for binding to DC-SIGN [58]. However, as discussed above, the binding of similar delipidated molecules such as capsular AM could depend on its supramolecular organization and exposure on the cell. In addition, the lack of exposure of LAM to the mycobacterial surface suggests that other Mtb mannosylated exposed ligands might be responsible for such interaction. Supporting this notion is the fact that mannan, another capsular polysaccharide, is able to block the binding of Mtb to DC-SIGN [113]. However, similarly to the scenario with CR3, studies including different mycobacterial strains displaying LAMs with distinct mannosylation patterns or strains deficient in terminal mannosylated LAM could not show any profound impact in the binding of Mtb to DC-SIGN [115].

In addition to mannose-capped LAM, Mtb's purified capsular α-glucans have been shown to function as potential ligands for DC-SIGN, and recognition by this lectin seems to be mediated by internal glycosyl residues of the polymer [114]. Since neither AM-deficient nor LAM-deficient strains were available at the time of this study (AM- and LAM-deficient mutants remain unavailable today), it is unclear to what extent the mentioned potential ligands each contribute to the DC-SIGN-mediated binding and phagocytosis by DC in the context of whole bacterial cells. Conversely, the recent development of an Mtb double mutant that is defective in capsular α-glucan and that has reduced virulence in mice [54] could help to understand the role of one of the major capsular components during the interaction of Mtb with host cells.

Role of nonpolysaccharide capsular antigens in the binding and uptake of Mtb by phagocytic cells

Capsular proteins

It was shown that the proteinaceous fraction of the Mtb capsule, and not the polysaccharide one, is responsible for the nonopsonic binding of Mtb to macrophages [116]. Subsequent studies demonstrated that most of the binding capacity of the Mtb capsule is associated with the chaperone GroEL2 and is mediated by the mammalian glycoprotein CD43 [117]. Discrepancies with previous studies regarding the contribution of the polysaccharide fraction of the capsule to the binding of Mtb by phagocytes could be related to the use of CHO cells expressing solely CR3 [87]. Additional studies have shown that other capsule-associated proteins could have a role in the binding of Mtb to phagocytes, such as the lipoprotein 19 kDa [118], the Ag85 complex [119] or the mycobacterial adhesion protein HbhA [120,121].

Capsular lipids

PDIMs, DATs and PIMs have been identified as the major Mtb capsule-associated lipids after glass-bead extraction of the capsule material [41]. Among them, the specific lipid PIM_2 has been implicated in the nonopsonic binding of Mtb to macrophages via CR3 [122]. Furthermore, an unidentified PIM species was shown to mediate the opsonic (possibly by the mannose-binding protein) and nonopsonic binding of Mtb to CHO cells through an identified receptor [108]. The ability of PIMs to mediate direct binding to CHO cells is inversely correlated to the abundance of the capsule, as it was demonstrated using Mtb clinical isolates with different degrees of encapsulation [87]. In addition, both high order PIMs (PIM5 and PIM6) were shown to bind to cell lines expression DC-SIGN [123].

Modulation of lung cell nonphagocytic functions by capsular components

The interaction of capsular components with nonphagocytic receptors has been shown to have an impact on other immune-related cellular process than phagocytosis. According to the current literature, Mtb capsular polysaccharides could induce both inhibitory and stimulatory effects when interacting with lung phagocytic cells. Mtb α-glucan appears to interfere with the complete maturation of monocyte-derived dendritic cells (MDDCs) by blocking CD1. CD1 molecules are prominently expressed by MDDCs and mediate presentation of lipid and glycolipid antigens to T cells, suggesting that α-glucan-stimulated DCs are deficient in presenting lipid antigens to T cells in a CD1-dependent manner [124]. In addition, capsular α-glucan has been shown to interact with DC-SIGN on DCs, modulating functionality by inducing the immunosuppressive cytokine IL-10 [114].

Early studies showed the capacity of H37Ra AM to inhibit lymphocytic functions *in vitro* [125]. Other immunological effects of AM have been reported in the cancer field by showing that AM from the Aoyama B strain could inhibit pulmonary metastasis by restoring the balance between Th1/Th2 cell response [126]. Of note, capsular AM and cell wall-associated LAM have some parallels in their immunomodulatory effect on macrophage and DCs. As previously commented, engagement of LAM with MR in human macrophages prevents phagosome–lysosome fusion [95]. It is well documented that the induction of IL-10 in LAM-stimulated macrophages is an inhibitory mechanism. LAM immunosuppression can also be achieved by its interaction with DC-SIGN and SIGNR3 [113,127,128]. Conversely, LAM can also induce a pro-inflammatory response [129] through its binding to the scavenger receptor CD36, leading to an enhancement of TNF production in LPS-stimulated macrophages [130]. It was recently demonstrated that LAM provides both anti- and pro-inflammatory signals when interacting with DCs via Dectin-2 [131].

The most recent proteomics analysis of *Mycobacterium* capsule demonstrated the presence of ESX-1 secreted proteins in *M. marinum* [38]. Capsular proteins such as ESAT-6 and CFP-10 have been implicated in the translocation of Mtb from the phagolysosome to the cytosol of myeloid cells [132]. However, these proteins could not be found in the Mtb capsule in the same analysis. Other capsule-associated proteins including the lipoprotein LpqH have been shown to induce apoptosis and the release of IL-1β during Mtb infection [133]. Of note, this lipoprotein has also been shown to interfere with the antigen presentation process in macrophages [134]. The proteolytic processing of GroEL2, another capsule-associated protein, has been suggested to contribute to the suboptimal antigen presentation during mycobacterial infection [135].

The role of capsule-associated lipids in modulating phagocyte responses has been extensively studied. Although no signaling receptor has been identified for capsular PDIMs, their presence in the mycobacterial cell envelope is associated with the protection against reactive nitrogen species [136]. Moreover, PDIM-deficient Mtb mutants are impaired in the infection of human macrophages [137]. Also, the association of PDIMs with the protein ESAT-6 to perform membrane damage in infected macrophages has been reported [138]. Several reports have demonstrated the recognition of PIMs by TLR-2 and TLR-4 [139] and their role in the induction of host granuloma-associated molecules including matrix metalloproteinase 9 (MMP-9) and cyclooxygenase 2 (Cox-2) [140]. A recent study demonstrated that the C-type lectin receptor DCAR in collaboration with FcRγ is an activated receptor for PIMs in monocyte-derived inflammatory cells promoting a protective Th1 immune response [141].

Antigenic variability associated with mycobacterial capsular polysaccharides

Many encapsulated bacteria have evolved to produce antigenically and chemically distinct capsule types (serogroups). There have been up to 80 different serogroups described in *E. coli* and 97 in the Gram-positive

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

bacterium *Streptococcus pneumoniae*. However, only a minor fraction of these serogroups accounts for the majority of invasive infections and consequently represent the main targets of conjugate vaccines. In the case of *Haemophilus influenzae*, among six identified capsular serotypes, type b is considered the most virulent [142]. Conversely, chemically identical capsular polysaccharides can be synthesized by different bacterial species [143]. The ability to classify isolates, based on differences in capsular polysaccharides, into serotypes is based on antibody reagents. Despite the fact that several reports have acknowledged the potential existence of Mtb surface antigenic variability associated with capsular polysaccharides, the concept of serogroup has never been explored in this pathogen in a global approach. It was reported that the Mtb AM-specific mAb 9d8 reacted differently to whole Mtb cells from a panel of 11 isolates from a hospital in the Bronx [71]. Results from a recent study highlighted the complexity of the antibody response against LAM and consequently the heterogeneity of the antigenic properties of this lipoglycan. Combination of different mAbs against LAM resulted in a more highly sensitive immunodetection assay capable of detecting LAM in urine of TB patients in the absence of HIV-1 coinfection [85]. Recently, differential recognition of isolated AM from a panel of 14 isolates representing six different lineages by an Mtb AM-specific polyclonal serum, generated through conjugate vaccination in mice, was demonstrated [112]. In that study, differential reactivity could be measured between isolates belonging to the same lineage, suggesting variability in the surface distribution of AM within the same geographic region. In another report, two Mtb H37Rv strains were shown to bind differently to CHO cells expressing CR3. The strain harboring a more prominent capsule showed an enhanced nonopsonic binding relative to the other strain [87], indicating that differences in capsular polysaccharides between encapsulated mycobacteria impact the recognition by phagocytic cells. Similarly, another study reported the altered capacity of two clinical isolates to bind human macrophages due to the production of truncated LAM [144]. These two isolates were drug-sensitive, despite the fact that the existence of a similar truncated LAM has previously been associated with ethambutol resistance [145]. This suggests that other mechanisms of generating structurally diverse surface polysaccharides different from the one induced by antitubercular drugs exist. Of note, the majority of mutations associated with the production of truncated LAM by Mtb have been shown to map to genes encoding arabinofuranosyltransferases, including EmbC or AftC [146,147].

Encapsulated bacteria generate capsule diversity through genomic variation associated with the polysaccharide biosynthetic locus. However, in Mtb, the genes associated with capsule synthesis are randomly distributed throughout the genome. A study including 4800 clinical isolates integrating homoplasic, non-synonymous and deleterious single-nucleotide polymorphisms associated with AM and α-glucan biosynthetic genes showed a high degree of clustering within the different genetic lineages (R.P.-R., unpublished data). Further experimental validation of such clusters in terms of AM and α-glucan chemical structure and reactivity to different AM and α-glucan specific antibody reagents will reveal the current picture of the capsule antigenic variability.

Another potential source of cell surface antigenic variability could be associated with the function of LrpG, which regulates the exposure of LAM to the cell surface [52,53].

Mechanisms of regulation of capsule production in Mtb

Little information is available about the ability of Mtb to modulate capsule production. Screening of a transposon mutant library identified several mutants in an ABC phosphate transporter gene with defects in capsule, indicating that phosphate could regulate capsular polysaccharide production [148]. Another report showed that the Esx-5-specific substrate PP10 is involved in capsule regulation in *M. marinum* [149]. At the level of maltose 1-phosphate synthesis as a building block for α-glucan production, the flux of intermediates through the two alternative pathways GlgC–GlgA and OtsA–OtsB–TreS–Pep2 might be subject to allosteric regulation of GlgC by phosphoenolpyruvate and glucose 6-phosphate as well as of OtsA by fructose 6-phosphate [150]. However, these observations rely on enzymatic *in vitro* characterizations only, so it is unclear whether these mechanisms play any role in regulating α-glucan biosynthesis in living cells. Activity of GlgE as the key enzyme in α-glucan synthesis is negatively regulated by phosphorylation via protein kinase PknB [151]. The occurrence of GlgE phosphorylation and its negative impact on overall conversion of maltose 1-phosphate to α-glucan has been observed *in vivo* in mycobacteria cells [151]. However, it is unknown what factors and conditions might control GlgE phosphorylation by PknB.

Targeting the capsule to the benefit of the host

Many successful preventive and therapeutic strategies against encapsulated bacteria rely on the use of polysaccharide-conjugate vaccines [152]. To properly cover the repertoire of clinically important serogroups,

such conjugate vaccines include several polysaccharides. For instance, the current anti-pneumococcal conjugate vaccine confers protection against 23 different serogroups [153]. The protection provided by polysaccharide-conjugate vaccines is known to be mediated by Abs, since capsular polysaccharides are well-known T-cell independent antigens. The concept of a vaccine against Mtb whose mechanism of protection is based on Abs has not been exploited and it is not currently contemplated in the TB vaccine development pipeline. In fact, all current vaccine candidates are immunologically similar in the sense that they promote an enhanced T-cell-mediated protection relative to BCG [154]. The idea of targeting the bacterial capsule through vaccination in Mtb has been previously attempted by several groups. Several LAM-derived AM conjugate vaccines were developed, including both Mtb-related (Ag85b) and unrelated (tetanus toxin) protein carriers and these vaccines demonstrated protective efficacy in different animal models of infection. Generation of AM-binding immunoglobulin G (IgG) and T-cell proliferation upon purified protein derivative (PPD) stimulation was associated with protection [155]. Similarly, an enhanced control of bacterial replication in lungs was registered during the first week of an aerosol Mtb infection in mice previously immunized with an AM (secreted and extracellular AM) conjugate including a secreted protein from *P. aeruginosa* [71]. AM conjugates have also been tested as a boost in BCG-primed mice using the intranasal route, demonstrating reduced lung inflammation after intravenous infection with Mtb [156]. More recently, it was demonstrated that the passive transfer of AM-immune serum from native capsular AM-Ag85b conjugate vaccinated mice to naïve mice prior to an aerosol infection enhances the control of bacterial dissemination to the spleen [112]. Similarly, adoptive transfer of T cells from the same mice showed a protective effect in both lungs and spleen. Animals immunized with AM-Ag85b lived significantly longer than BCG-vaccinated mice. This suggests that both antibody and T-cell-mediated protection is key to provide a more efficient protection and validate previous conjugate vaccine approaches targeting the Mtb capsule. There is a single report on α-glucan conjugate vaccine, but no protection data were derived from such study [53].

Several groups have demonstrated that administration of mAbs with specificity for capsular AM or LAM can modify the course of experimental mycobacterial infection in mice to the benefit of the host [155,157]. Moreover, we have recently demonstrated an association between the presence of AM-binding IgGs in human sera and the capacity of such sera to perform opsonophagocytosis and reduce Mtb intracellular growth in human macrophages [111]. Regarding the role of Abs in natural protection against Mtb, one recent report showed the production of protective Abs by latently and uninfected healthcare workers exposed to TB with specificity for surface exposed proteins [158]. Interestingly, one of these proteins (Rv0242c) was previously found in the capsule of Mtb [38].

As the capsule is the outermost compartment of the cell wall, any strategy targeting the capsule may interfere with the initial interaction of the bacterium with the host. In the case of Mtb, this is particularly important since this pathogen has developed immune evasion strategies to remain alive inside the host despite a fully functioning immune system. Encapsulated Mtb can access host cells in the absence of opsonins, which favors bacterial survival inside host cells. Conversely, an antibacterial response is activated in infected host cells when this entry occurs in the presence of opsonins, such as immunoglobulins. Consequently, a protective vaccine directed to the capsule could promote the targeting of the bacterium to elimination.

Concluding remarks

The capsule of Mtb is the outermost structure of the tubercle bacillus and has a significant impact on the initial infection program. The evolution of the concept of the Mtb capsule has been determined by key seminal works beginning with early observations of a surrounding layer on the host-grown species of *M. leprae* and *M. lepramurium* [29], to the subsequent studies on other mycobacterial species, including Mtb, that were accompanied with improvements in both sample preparation and technological advances in electron microscopy [28,31,33,36,38,82]. Subsequent biochemical studies to determine the molecular composition of the capsule demonstrated that the Mtb capsule is weakly bound to the cell wall and is mostly composed of polysaccharides [26,43]. More importantly, it was determined that the capsule is easily detached from the cell by agitation or by the presence of detergents, suggesting that culturing Mtb with detergent could render the bacterium unencapsulated, leaving other cell-wall-associated components exposed. Although many of the controversies regarding the role of some cell-wall components, including LAM, in the mediation of uptake and activation of host cells [94,95,101] upon interaction with Mtb could be explained by the absence of the capsule, it is important to highlight that the mycobacterial cell envelope is heterogeneous and dynamic (Figure 1). Consequently, we cannot rule out that LAM could be exposed at some regions of the cell and masked in others when the

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

capsule is present. The study of the heterogeneity in the dynamics of the Mtb cell envelope is an important area of research that should be expanded and could offer some clues about the relative role of each of the surface components during infection. The relatively recent development of mycobacterial genetics, particularly the ability to generate precisely defined mutants that delete one or more specific pathways, provides unprecedented opportunities to dissect these systems [50]. More work needs to be done to determine how α-glucan reaches the extracellular space and how it is linked to the cell wall. The same is true for AM. Although it is clear that LAM and AM follow similar biosynthetic pathways, the divergent final steps are still unknown. How Mtb spatially organizes capsular components is still an open question and understanding this will help to define the concept of surface antigenic variability, which has been suggested in the field but never investigated using a global approach. The fact that this compartment can be targeted through vaccines [21,112] suggests that it has an important role during infection. In summary, improved understanding of capsule biogenesis in Mtb will illuminate part of the physiological complexity of this bacterium and will contribute to the development of novel anti-infective strategies.

Abbreviations

ABC, ATP-binding cassette; Abs, antibodies; AGP, peptidoglycan–arabinogalactan complex; AM, D-arabino-D-mannan; BMDMs, bone marrow-derived macrophages; BMMs, bone marrow macrophages; CHO, Chinese hamster ovary; cryo-EM, cryo-electron microscopy; DATs, diacyl trehaloses; DBE, debranching enzyme; DCs, dendritic cells; EPS, exopolysaccharides; ESX-1, ESAT-6 secretion system-1; EVs, extracellular vesicles; FcRs, Fc receptors; GB, glass beads; GH, glycoside hydrolase; GP, glycogen phosphorylase; GPL, glycopeptidolipid; GS, glycogen synthase; GTs, glycosyltransferases; HMDM, human monocyte-derived macrophages; ICAM, intercellular adhesion molecule; IM, inner membrane; LAM, lipoarabinomannan; LM, lipomannan; mAbs, monoclonal antibodies; MBL, mannose-binding lectin; MDDCs, monocyte-derived dendritic cells; MMP-9, matrix metalloproteinase 9; MR, mannose receptor; MTX, 5-methylthio-d-xylofuranose; OM, outer membrane; PDIMs, phthiocerol dimycocerosates; PE, phosphatidylethanolamine; PGA, poly-γ-glutamate; PILAM, phosphatidyl-myo-inositol capped lipoarabinomannan; PIM_4, phosphatidyl inositol mannoside 4; PIMs, phosphatidyl-*myo*-inositol mannosides; PPD, purified protein derivative; SEM, scanning electron micrograph; SGL, sulfoglycolipids; TB, tuberculosis.

Author Contribution

M.E.G. and R.P.-R. conceived the idea. R.K., M.E.G. and R.P.-R. design the manuscript outline and wrote the manuscript with support of A.P., I.A., J.C., J.A. and W.R.J.

Acknowledgements

R.P.-R. is supported in part by NIH/NIAID grant AI115091. R.P.-R. is further a 'Ramon y Cajal' fellow from the Spanish Ministry of Economy and Competitiveness. R.P.-R. and M.E.G. acknowledge supports by MINECO/FEDER EU contracts SAF2016-77433-R, BFU2016-77427-C2-2-R and BFU2017-92223-EXP, respectively. R.P.-R. and M.E.G. acknowledge support from CIC bioGUNE through the Severo Ochoa Excellence Accreditation SEV-2016-0644. R.K. acknowledges support from the Jürgen Manchot Foundation. A.P. and I.A. acknowledge the support of the Basque Government.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Moxon, E.R. and Kroll, J.S. (1990) The role of bacterial polysaccharide capsules as virulence factors. *Curr. Top. Microbiol. Immunol.* **150**, 65–85 PMID:2404690

2 Wen, Z. and Zhang, J. (2015) Bacterial capsules. In *Molecular Medical Microbiology*, Vol. 1, pp. 33–53, Elsevier https://doi.org/10.1016/B978-0-12-397169-2.00003-2

3 Smith, W.D., Bardin, E., Cameron, L., Edmondson, C.L., Farrant, K.V., Martin, I. et al. (2017) Current and future therapies for *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. *FEMS Microbiol. Lett.* **364**, fnx121 https://doi.org/10.1093/femsle/fnx121

4 Taylor, C.M. and Roberts, I.S. (2005) Capsular polysaccharides and their role in virulence. *Contrib. Microbiol.* **12**, 55–66 https://doi.org/10.1159/000081689

5 Costerton, J.W., Irvin, R.T. and Cheng, K.J. (1981) The bacterial glycocalyx in nature and disease. *Annu. Rev. Microbiol.* **35**, 299–324 https://doi.org/10.1146/annurev.mi.35.100181.001503

6 Beveridge, T. J., Lawrence, J.R. and Murray, R.G.E. (2007) Sampling and staining for light microscopy. In *Methods for General and Molecular Microbiology* (Press, A., ed.), pp. 19–33, ASM Press, Washington, DC
7 Anderson, K.L. (1998) Cationized ferritin as a stain for electron microscopic observation of bacterial ultrastructure. *Biotech. Histochem.* **73**, 278–288 https://doi.org/10.3109/10520299809141121
8 Dubochet, J., McDowall, A.W., Menge, B., Schmid, E.N. and Lickfeld, K.G. (1983) Electron microscopy of frozen-hydrated bacteria. *J. Bacteriol.* **155**, 381–390 PMID:6408064
9 Graham, L.L., Harris, R., Villiger, W. and Beveridge, T.J. (1991) Freeze-substitution of gram-negative eubacteria: general cell morphology and envelope profiles. *J. Bacteriol.* **173**, 1623–1633 https://doi.org/10.1128/jb.173.5.1623-1633.1991
10 McIntosh, J.R. (2007) *Cellular Electron Microscopy*, Elsevier, San Diego, CA
11 Rendueles, O., Garcia-Garcera, M., Neron, B., Touchon, M. and Rocha, E.P.C. (2017) Abundance and co-occurrence of extracellular capsules increase environmental breadth: implications for the emergence of pathogens. *PLoS Pathog.* **13**, e1006525 https://doi.org/10.1371/journal.ppat.1006525
12 Whitfield, C. (2006) Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu. Rev. Biochem.* **75**, 39–68 https://doi.org/10.1146/annurev.biochem.75.103004.142545
13 Cuthbertson, L., Kos, V. and Whitfield, C. (2010) ABC transporters involved in export of cell surface glycoconjugates. *Microbiol. Mol. Biol. Rev.* **74**, 341–362 https://doi.org/10.1128/MMBR.00009-10
14 Peleg, A., Shifrin, Y., Ilan, O., Nadler-Yona, C., Nov, S., Koby, S. et al. (2005) Identification of an *Escherichia coli* operon required for formation of the O-antigen capsule. *J. Bacteriol.* **187**, 5259–5266 https://doi.org/10.1128/JB.187.15.5259-5266.2005
15 Martens, E.C., Roth, R., Heuser, J.E. and Gordon, J.I. (2009) Coordinate regulation of glycan degradation and polysaccharide capsule biosynthesis by a prominent human gut symbiont. *J. Biol. Chem.* **284**, 18445–18457 https://doi.org/10.1074/jbc.M109.008094
16 Whitfield, G.B., Marmont, L.S. and Howell, P.L. (2015) Enzymatic modifications of exopolysaccharides enhance bacterial persistence. *Front. Microbiol.* **6**, 471 https://doi.org/10.3389/fmicb.2015.00471
17 Cavallari, M. and De Libero, G. (2017) From immunologically archaic to neoteric glycovaccines. *Vaccines (Basel)* **5**, E4 https://doi.org/10.3390/vaccines5010004
18 Ophir, T. and Gutnick, D.L. (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. *Appl. Environ. Microbiol.* **60**, 740–745 PMID:16349202
19 Yoshida, K., Matsumoto, T., Tateda, K., Uchida, K., Tsujimoto, S., Iwakurai, Y. et al. (2001) Protection against pulmonary infection with *Klebsiella pneumoniae* in mice by interferon-gamma through activation of phagocytic cells and stimulation of production of other cytokines. *J. Med. Microbiol.* **50**, 959–964 https://doi.org/10.1099/0022-1317-50-11-959
20 WHO. (2017) *Global Tuberculosis Report 2017*, World Health Organization, Geneva, Switzerland
21 Angala, S.K., Belardinelli, J.M., Huc-Claustre, E., Wheat, W.H. and Jackson, M. (2014) The cell envelope glycoconjugates of *Mycobacterium tuberculosis*. *Crit. Rev. Biochem. Mol. Biol.* **49**, 361–399 https://doi.org/10.3109/10409238.2014.925420
22 Jankute, M., Cox, J.A., Harrison, J. and Besra, G.S. (2015) Assembly of the mycobacterial cell wall. *Annu. Rev. Microbiol.* **69**, 405–423 https://doi.org/10.1146/annurev-micro-091014-104121
23 Brennan, P.J. and Crick, D.C. (2007) The cell-wall core of *Mycobacterium tuberculosis* in the context of drug discovery. *Curr. Top. Med. Chem.* **7**, 475–488 https://doi.org/10.2174/156802607780059763
24 Daffe, M., Reyrat, J.M. and Avenir, G. (2008) *The Mycobacterial Cell Envelope*, ASM
25 Lemassu, A., Ortalo-Magne, A., Bardou, F., Silve, G., Laneelle, M.A. and Daffe, M. (1996) Extracellular and surface-exposed polysaccharides of non-tuberculous mycobacteria. *Microbiology* **142** (Pt 6), 1513–1520 https://doi.org/10.1099/13500872-142-6-1513
26 Ortalo-Magne, A., Dupont, M.A., Lemassu, A., Andersen, A.B., Gounon, P. and Daffe, M. (1995) Molecular composition of the outermost capsular material of the tubercle bacillus. *Microbiology* **141** (Pt 7), 1609–1620 https://doi.org/10.1099/13500872-141-7-1609
27 Hanks, J.H. (1961) Capsules in electron micrographs of *Mycobacterium leprae*. *Int. J. Lepr.* **29**, 84–87 PMID:13711244
28 Werner, G.H. (1951) Electron-microscopic studies on the cellular morphology of tubercle bacilli. *Bibl. Tuberc.* **5**, 53–90 PMID:13018094
29 Brieger, E.M. and Glauert, A.M. (1956) Sporelike structures in the tubercle bacillus. *Nature* **178**, 544 https://doi.org/10.1038/178544a0
30 Yamamoto, T., Nishiura, M., Harada, N. and Imaeda, T. (1958) Electron microscopy of ultra-thin sections of lepra cells and *Mycobacterium leprae*. *Int. J. Lepr.* **26**, 1–8 PMID:13586951
31 Chapman, G.B., Hanks, J.H. and Wallace, J.H. (1959) An electron microscope study of the disposition and fine structure of *Mycobacterium lepraemurium* in mouse spleen. *J. Bacteriol.* **77**, 205–211 PMID:13630872
32 Daffe, M. and Etienne, G. (1999) The capsule of *Mycobacterium tuberculosis* and its implications for pathogenicity. *Tuber. Lung Dis.* **79**, 153–169 https://doi.org/10.1054/tuld.1998.0200
33 Hanks, J.H. (1961) Significance of capsular components of *Mycobacterium leprae* and other mycobacteria. *Int. J. Lepr.* **29**, 74–83 PMID:13711245
34 Frehel, C. and Rastogi, N. (1987) *Mycobacterium leprae* surface components intervene in the early phagosome-lysosome fusion inhibition event. *Infect. Immun.* **55**, 2916–2921 PMID:3316028
35 Draper, P. and Rees, R.J. (1970) Electron-transparent zone of mycobacteria may be a defence mechanism. *Nature* **228**, 860–861 https://doi.org/10.1038/228860a0
36 Frehel, C., Ryter, A., Rastogi, N. and David, H. (1986) The electron-transparent zone in phagocytized *Mycobacterium avium* and other mycobacteria: formation, persistence and role in bacterial survival. *Ann. Inst. Pasteur. Microbiol.* **137B**, 239–257 https://doi.org/10.1016/S0769-2609(86)80115-6
37 Paul, T.R. and Beveridge, T.J. (1992) Reevaluation of envelope profiles and cytoplasmic ultrastructure of mycobacteria processed by conventional embedding and freeze-substitution protocols. *J. Bacteriol.* **174**, 6508–6517 https://doi.org/10.1128/jb.174.20.6508-6517.1992
38 Sani, M., Houben, E.N., Geurtsen, J., Pierson, J., de Punder, K., van Zon, M. et al. (2010) Direct visualization by cryo-EM of the mycobacterial capsular layer: a labile structure containing ESX-1-secreted proteins. *PLoS Pathog.* **6**, e1000794 https://doi.org/10.1371/journal.ppat.1000794
39 Rastogi, N., Rauzier, J.Y., Papa, F.P. and David, H.L. (1986) Biochemical and cultural analysis of mycobacterial recombinants obtained by spheroplast fusion. *Ann. Inst. Pasteur. Microbiol.* **137A**, 135–142 https://doi.org/10.1016/S0769-2609(86)80018-7
40 Picard, B., Frehel, C. and Rastogi, N. (1984) Cytochemical characterization of mycobacterial outer surfaces. *Acta Leprol.* **2**, 227–235 PMID:6398583

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

41 Ortalo-Magne, A., Lemassu, A., Laneelle, M.A., Bardou, F., Silve, G., Gounon, P. et al. (1996) Identification of the surface-exposed lipids on the cell envelopes of *Mycobacterium tuberculosis* and other mycobacterial species. *J. Bacteriol.* **178**, 456–461 https://doi.org/10.1128/jb.178.2.456-461.1996
42 Dinadayala, P., Sambou, T., Daffe, M. and Lemassu, A. (2008) Comparative structural analyses of the α-glucan and glycogen from *Mycobacterium bovis*. *Glycobiology* **18**, 502–508 https://doi.org/10.1093/glycob/cwn031
43 Lemassu, A. and Daffe, M. (1994) Structural features of the exocellular polysaccharides of *Mycobacterium tuberculosis*. *Biochem. J.* **297** (Pt 2), 351–357 https://doi.org/10.1042/bj2970351
44 Sambou, T., Dinadayala, P., Stadthagen, G., Barilone, N., Bordat, Y., Constant, P. et al. (2008) Capsular glucan and intracellular glycogen of *Mycobacterium tuberculosis*: biosynthesis and impact on the persistence in mice. *Mol. Microbiol.* **70**, 762–774 https://doi.org/10.1111/j.1365-2958.2008.06445.x
45 Preiss, J. (2009) Glycogen: biosynthesis and regulation. *EcoSal Plus.* **3**. https://doi.org/10.1128/ecosalplus.4.7.4
46 Cifuente, J.O., Comino, N., Madariaga-Marcos, J., Lopez-Fernandez, S., Garcia-Alija, M., Agirre, J. et al. (2016) Structural basis of glycogen biosynthesis regulation in bacteria. *Structure* **24**, 1613–1622 https://doi.org/10.1016/j.str.2016.06.023
47 Buschiazzo, A., Ugalde, J.E., Guerin, M.E., Shepard, W., Ugalde, R.A. and Alzari, P.M. (2004) Crystal structure of glycogen synthase: homologous enzymes catalyze glycogen synthesis and degradation. *EMBO J.* **23**, 3196–3205 https://doi.org/10.1038/sj.emboj.7600324
48 Abad, M.C., Binderup, K., Rios-Steiner, J., Arni, R.K., Preiss, J. and Geiger, J.H. (2002) The X-ray crystallographic structure of *Escherichia coli* branching enzyme. *J. Biol. Chem.* **277**, 42164–42170 https://doi.org/10.1074/jbc.M205746200
49 Montero, M., Almagro, G., Eydallin, G., Viale, A.M., Munoz, F.J., Bahaji, A. et al. (2011) *Escherichia coli* glycogen genes are organized in a single glgBXCAP transcriptional unit possessing an alternative suboperonic promoter within glgC that directs glgAP expression. *Biochem. J.* **433**, 107–117 https://doi.org/10.1042/BJ20101186
50 Rashid, A.M., Batey, S.F., Syson, K., Koliwer-Brandl, H., Miah, F., Barclay, J.E. et al. (2016) Assembly of α-glucan by GlgE and GlgB in mycobacteria and streptomycetes. *Biochemistry* **55**, 3270–3284 https://doi.org/10.1021/acs.biochem.6b00209
51 Kalscheuer, R., Syson, K., Veeraraghavan, U., Weinrick, B., Biermann, K.E., Liu, Z. et al. (2010) Self-poisoning of *Mycobacterium tuberculosis* by targeting GlgE in an α-glucan pathway. *Nat. Chem. Biol.* **6**, 376–384 https://doi.org/10.1038/nchembio.340
52 Albesa-Jove, D. and Guerin, M.E. (2016) The conformational plasticity of glycosyltransferases. *Curr. Opin. Struct. Biol.* **40**, 23–32 https://doi.org/10.1016/j.sbi.2016.07.007
53 Schwebach, J.R., Glatman-Freedman, A., Gunther-Cummins, L., Dai, Z., Robbins, J.B., Schneerson, R. et al. (2002) Glucan is a component of the *Mycobacterium tuberculosis* surface that is expressed in vitro and in vivo. *Infect. Immun.* **70**, 2566–2575 https://doi.org/10.1128/IAI.70.5.2566-2575.2002
54 Koliwer-Brandl, H., Syson, K., van de Weerd, R., Chandra, G., Appelmelk, B., Alber, M. et al. (2016) Metabolic network for the biosynthesis of intra- and extracellular α-glucans required for virulence of *Mycobacterium tuberculosis*. *PLoS Pathog.* **12**, e1005768 https://doi.org/10.1371/journal.ppat.1005768
55 Gest, P., Kaur, D., Pham, H.T., van der Woerd, M., Hansen, E., Brennan, P.J. et al. (2008) Preliminary crystallographic analysis of GpgS, a key glucosyltransferase involved in methylglucose lipopolysaccharide biosynthesis in *Mycobacterium tuberculosis*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **64**, 1121–1124 https://doi.org/10.1107/S1744309108032892
56 Albesa-Jove, D., Romero-Garcia, J., Sancho-Vaello, E., Contreras, F.X., Rodrigo-Unzueta, A., Comino, N. et al. (2017) Structural snapshots and loop dynamics along the catalytic cycle of glycosyltransferase GpgS. *Structure* **25**, 1034–1044.e3 https://doi.org/10.1016/j.str.2017.05.009
57 Maranha, A., Moynihan, P.J., Miranda, V., Correia Lourenco, E., Nunes-Costa, D., Fraga, J.S. et al. (2015) Octanoylation of early intermediates of mycobacterial methylglucose lipopolysaccharides. *Sci. Rep.* **5**, 13610 https://doi.org/10.1038/srep13610
58 Maeda, N., Nigou, J., Herrmann, J.L., Jackson, M., Amara, A., Lagrange, P.H. et al. (2003) The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J. Biol. Chem.* **278**, 5513–5516 https://doi.org/10.1074/jbc.C200586200
59 Treumann, A., Xidong, F., McDonnell, L., Derrick, P.J., Ashcroft, A.E., Chatterjee, D. et al. (2002) 5-Methylthiopentose: a new substituent on lipoarabinomannan in *Mycobacterium tuberculosis*. *J. Mol. Biol.* **316**, 89–100 https://doi.org/10.1006/jmbi.2001.5317
60 Turnbull, W.B., Shimizu, K.H., Chatterjee, D., Homans, S.W. and Treumann, A. (2004) Identification of the 5-methylthiopentosyl substituent in *Mycobacterium tuberculosis* lipoarabinomannan. *Angew. Chem. Int. Ed. Engl.* **43**, 3918–3922 https://doi.org/10.1002/anie.200454119
61 Sigal, G.B., Pinter, A., Lowary, T.L., Kawasaki, M., Li, A., Mathew, A. et al. (2018) A novel sensitive immunoassay targeting the 5-methylthio-d-xylofuranose-lipoarabinomannan epitope meets the WHO's performance target for tuberculosis diagnosis. *J. Clin. Microbiol.* **56**, e01338-18 https://doi.org/10.1128/JCM.01338-18
62 Pitarque, S., Larrouy-Maumus, G., Payre, B., Jackson, M., Puzo, G. and Nigou, J. (2008) The immunomodulatory lipoglycans, lipoarabinomannan and lipomannan, are exposed at the mycobacterial cell surface. *Tuberculosis (Edinb)* **88**, 560–565 https://doi.org/10.1016/j.tube.2008.04.002
63 Hoffmann, C., Leis, A., Niederweis, M., Plitzko, J.M. and Engelhardt, H. (2008) Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. *Proc. Natl Acad. Sci. U.S.A.* **105**, 3963–3967 https://doi.org/10.1073/pnas.0709530105
64 Zuber, B., Chami, M., Houssin, C., Dubochet, J., Griffiths, G. and Daffe, M. (2008) Direct visualization of the outer membrane of mycobacteria and corynebacteria in their native state. *J. Bacteriol.* **190**, 5672–5680 https://doi.org/10.1128/JB.01919-07
65 Dmitriev, B.A., Ehlers, S., Rietschel, E.T. and Brennan, P.J. (2000) Molecular mechanics of the mycobacterial cell wall: from horizontal layers to vertical scaffolds. *Int. J. Med. Microbiol.* **290**, 251–258 https://doi.org/10.1016/S1438-4221(00)80122-8
66 Shukla, S., Richardson, E.T., Athman, J.J., Shi, L., Wearsch, P.A., McDonald, D. et al. (2014) *Mycobacterium tuberculosis* lipoprotein LprG binds lipoarabinomannan and determines its cell envelope localization to control phagolysosomal fusion. *PLoS Pathog.* **10**, e1004471 https://doi.org/10.1371/journal.ppat.1004471
67 Gaur, R.L., Ren, K., Blumenthal, A., Bhamidi, S., Gonzalez-Nilo, F.D., Jackson, M. et al. (2014) LprG-mediated surface expression of lipoarabinomannan is essential for virulence of *Mycobacterium tuberculosis*. *PLoS Pathog.* **10**, e1004376 https://doi.org/10.1371/journal.ppat.1004376
68 Prados-Rosales, R., Baena, A., Martinez, L.R., Luque-Garcia, J., Kalscheuer, R., Veeraraghavan, U. et al. (2011) Mycobacteria release active membrane vesicles that modulate immune responses in a TLR2-dependent manner in mice. *J. Clin. Invest.* **121**, 1471–1483 https://doi.org/10.1172/JCI44261

69 Brown, L., Wolf, J.M., Prados-Rosales, R. and Casadevall, A. (2015) Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nat. Rev. Microbiol.* **13**, 620–630 https://doi.org/10.1038/nrmicro3480
70 Yang, L., Sinha, T., Carlson, T.K., Keiser, T.L., Torrelles, J.B. and Schlesinger, L.S. (2013) Changes in the major cell envelope components of *Mycobacterium tuberculosis* during in vitro growth. *Glycobiology* **23**, 926–934 https://doi.org/10.1093/glycob/cwt029
71 Glatman-Freedman, A., Casadevall, A., Dai, Z., Jacobs, W.R., Li, A., Morris, S.L. et al. (2004) Antigenic evidence of prevalence and diversity of *Mycobacterium tuberculosis* arabinomannan. *J. Clin. Microbiol.* **42**, 3225–3231 https://doi.org/10.1128/JCM.42.7.3225-3231.2004
72 Raynaud, C., Etienne, G., Peyron, P., Laneelle, M.A. and Daffe, M. (1998) Extracellular enzyme activities potentially involved in the pathogenicity of *Mycobacterium tuberculosis*. *Microbiology* **144** (Pt 2), 577–587 https://doi.org/10.1099/00221287-144-2-577
73 Majlessi, L., Prados-Rosales, R., Casadevall, A. and Brosch, R. (2015) Release of mycobacterial antigens. *Immunol. Rev.* **264**, 25–45 https://doi.org/10.1111/imr.12251
74 Wong, K. (2017) The role of ESX-1 in *Mycobacterium tuberculosis* pathogenesis. *Microbiol. Spectr.* **5**. https://doi.org/10.1128/microbiolspec.TBTB2-0001-2015
75 Armstrong, J.A. and Hart, P.D. (1975) Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J. Exp. Med.* **142**, 1–16 https://doi.org/10.1084/jem.142.1.1
76 Ernst, J.D. (1998) Macrophage receptors for *Mycobacterium tuberculosis*. *Infect. Immun.* **66**, 1277–1281 PMID:9529042
77 Torrelles, J.B. and Schlesinger, L.S. (2017) Integrating lung physiology, immunology, and tuberculosis. *Trends Microbiol.* **25**, 688–697 https://doi.org/10.1016/j.tim.2017.03.007
78 Cambi, A. and Figdor, C.G. (2005) Levels of complexity in pathogen recognition by C-type lectins. *Curr. Opin. Immunol.* **17**, 345–351 https://doi.org/10.1016/j.coi.2005.05.011
79 Venkataswamy, M.M., Goldberg, M.F., Baena, A., Chan, J., Jacobs, Jr, W.R. and Porcelli, S.A. (2012) In vitro culture medium influences the vaccine efficacy of *Mycobacterium bovis* BCG. *Vaccine* **30**, 1038–1049 https://doi.org/10.1016/j.vaccine.2011.12.044
80 Prados-Rosales, R., Carreno, L.J., Weinrick, B., Batista-Gonzalez, A., Glatman-Freedman, A., Xu, J. et al. (2016) The type of growth medium affects the presence of a mycobacterial capsule and is associated with differences in protective efficacy of BCG vaccination against *Mycobacterium tuberculosis*. *J. Infect. Dis.* **214**, 426–437 https://doi.org/10.1093/infdis/jiw153
81 Daffe, M. and Draper, P. (1998) The envelope layers of mycobacteria with reference to their pathogenicity. *Adv. Microb. Physiol.* **39**, 131–203 https://doi.org/10.1016/S0065-2911(08)60016-8
82 Stokes, R.W., Norris-Jones, R., Brooks, D.E., Beveridge, T.J., Doxsee, D. and Thorson, L.M. (2004) The glycan-rich outer layer of the cell wall of *Mycobacterium tuberculosis* acts as an antiphagocytic capsule limiting the association of the bacterium with macrophages. *Infect. Immun.* **72**, 5676–5686 https://doi.org/10.1128/IAI.72.10.5676-5686.2004
83 Kallenius, G., Correia-Neves, M., Buteme, H., Hamasur, B. and Svenson, S.B. (2016) Lipoarabinomannan, and its related glycolipids, induce divergent and opposing immune responses to *Mycobacterium tuberculosis* depending on structural diversity and experimental variations. *Tuberculosis (Edinb)* **96**, 120–130 https://doi.org/10.1016/j.tube.2015.09.005
84 Zheng, R.B., Jegouzo, S.A.F., Joe, M., Bai, Y., Tran, H.A., Shen, K. et al. (2017) Insights into interactions of mycobacteria with the host innate immune system from a novel array of synthetic mycobacterial glycans. *ACS Chem. Biol.* **12**, 2990–3002 https://doi.org/10.1021/acschembio.7b00797
85 Choudhary, A., Patel, D., Honnen, W., Lai, Z., Prattipati, R.S., Zheng, R.B. et al. (2018) Characterization of the antigenic heterogeneity of lipoarabinomannan, the major surface glycolipid of *Mycobacterium tuberculosis*, and complexity of antibody specificities toward this antigen. *J. Immunol.* **200**, 3053–3066 https://doi.org/10.4049/jimmunol.1701673
86 Cywes, C., Godenir, N.L., Hoppe, H.C., Scholle, R.R., Steyn, L.M., Kirsch, R.E. et al. (1996) Nonopsonic binding of *Mycobacterium tuberculosis* to human complement receptor type 3 expressed in Chinese hamster ovary cells. *Infect. Immun.* **64**, 5373–5383 PMID:8945590
87 Cywes, C., Hoppe, H.C., Daffe, M. and Ehlers, M.R. (1997) Nonopsonic binding of *Mycobacterium tuberculosis* to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. *Infect. Immun.* **65**, 4258–4266 PMID:9317035
88 Hu, C., Mayadas-Norton, T., Tanaka, K., Chan, J. and Salgame, P. (2000) *Mycobacterium tuberculosis* infection in complement receptor 3-deficient mice. *J. Immunol.* **165**, 2596–2602 https://doi.org/10.4049/jimmunol.165.5.2596
89 Melo, M.D., Catchpole, I.R., Haggar, G. and Stokes, R.W. (2000) Utilization of CD11b knockout mice to characterize the role of complement receptor 3 (CR3, CD11b/CD18) in the growth of *Mycobacterium tuberculosis* in macrophages. *Cell Immunol.* **205**, 13–23 https://doi.org/10.1006/cimm.2000.1710
90 Rooyakkers, A.W. and Stokes, R.W. (2005) Absence of complement receptor 3 results in reduced binding and ingestion of *Mycobacterium tuberculosis* but has no significant effect on the induction of reactive oxygen and nitrogen intermediates or on the survival of the bacteria in resident and interferon-γ activated macrophages. *Microb. Pathog.* **39**, 57–67 https://doi.org/10.1016/j.micpath.2005.05.001
91 Hirsch, C.S., Ellner, J.J., Russell, D.G. and Rich, E.A. (1994) Complement receptor-mediated uptake and tumor necrosis factor-α-mediated growth inhibition of *Mycobacterium tuberculosis* by human alveolar macrophages. *J. Immunol.* **152**, 743–753 PMID:8283049
92 Watford, W.T., Ghio, A.J. and Wright, J.R. (2000) Complement-mediated host defense in the lung. *Am. J. Physiol. Lung Cell Mol. Physiol.* **279**, L790–L798 https://doi.org/10.1152/ajplung.2000.279.5.L790
93 Zimmerli, S., Edwards, S. and Ernst, J.D. (1996) Selective receptor blockade during phagocytosis does not alter the survival and growth of *Mycobacterium tuberculosis* in human macrophages. *Am. J. Respir. Cell Mol. Biol.* **15**, 760–770 https://doi.org/10.1165/ajrcmb.15.6.8969271
94 Schlesinger, L.S. (1993) Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. *J. Immunol.* **150**, 2920–2930 PMID:8454864
95 Kang, P.B., Azad, A.K., Torrelles, J.B., Kaufman, T.M., Beharka, A., Tibesar, E. et al. (2005) The human macrophage mannose receptor directs *Mycobacterium tuberculosis* lipoarabinomannan-mediated phagosome biogenesis. *J. Exp. Med.* **202**, 987–999 https://doi.org/10.1084/jem.20051239
96 Rajaram, M.V.S., Arnett, E., Azad, A.K., Guirado, E., Ni, B., Gerberick, A.D. et al. (2017) *M. tuberculosis*-initiated human mannose receptor signaling regulates macrophage recognition and vesicle trafficking by FcRγ-Chain, Grb2, and SHP-1. *Cell Rep.* **21**, 126–140 https://doi.org/10.1016/j.celrep.2017.09.034
97 Nigou, J., Zelle-Rieser, C., Gilleron, M., Thurnher, M. and Puzo, G. (2001) Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J. Immunol.* **166**, 7477–7485 https://doi.org/10.4049/jimmunol.166.12.7477

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

98 Astarie-Dequeker, C., N'Diaye, E.N., Le Cabec, V., Rittig, M.G., Prandi, J. and Maridonneau-Parini, I. (1999) The mannose receptor mediates uptake of pathogenic and nonpathogenic mycobacteria and bypasses bactericidal responses in human macrophages. *Infect. Immun.* **67**, 469–477 PMID:9916047
99 Mokoena, T. and Gordon, S. (1985) Human macrophage activation. Modulation of mannosyl, fucosyl receptor activity in vitro by lymphokines, gamma and alpha interferons, and dexamethasone. *J. Clin. Invest.* **75**, 624–631 https://doi.org/10.1172/JCI111740
100 Chroneos, Z. and Shepherd, V.L. (1995) Differential regulation of the mannose and SP-A receptors on macrophages. *Am. J. Physiol.* **269**, L721–L726 https://doi.org/10.1152/ajplung.1995.269.6.L721
101 Torrelles, J.B., Azad, A.K., Henning, L.N., Carlson, T.K. and Schlesinger, L.S. (2008) Role of C-type lectins in mycobacterial infections. *Curr. Drug Targets* **9**, 102–112 https://doi.org/10.2174/138945008783502467
102 Weikert, L.F., Edwards, K., Chroneos, Z.C., Hager, C., Hoffman, L. and Shepherd, V.L. (1997) SP-A enhances uptake of bacillus Calmette-Guerin by macrophages through a specific SP-A receptor. *Am. J. Physiol.* **272**, L989–L995 https://doi.org/10.1152/ajpcell.1997.272.3.C989
103 Gaynor, C.D., McCormack, F.X., Voelker, D.R., McGowan, S.E. and Schlesinger, L.S. (1995) Pulmonary surfactant protein A mediates enhanced phagocytosis of *Mycobacterium tuberculosis* by a direct interaction with human macrophages. *J. Immunol.* **155**, 5343–5351 PMID:7594549
104 Sidobre, S., Nigou, J., Puzo, G. and Riviere, M. (2000) Lipoglycans are putative ligands for the human pulmonary surfactant protein A attachment to mycobacteria. Critical role of the lipids for lectin-carbohydrate recognition. *J. Biol. Chem.* **275**, 2415–2422 https://doi.org/10.1074/jbc.275.4.2415
105 Ferguson, J.S., Voelker, D.R., McCormack, F.X. and Schlesinger, L.S. (1999) Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J. Immunol.* **163**, 312–321 PMID:10384130
106 Taylor, M.E. and Drickamer, K. (1993) Structural requirements for high affinity binding of complex ligands by the macrophage mannose receptor. *J. Biol. Chem.* **268**, 399–404 PMID:8416946
107 Polotsky, V.Y., Belisle, J.T., Mikusova, K., Ezekowitz, R.A. and Joiner, K.A. (1997) Interaction of human mannose-binding protein with *Mycobacterium avium*. *J. Infect. Dis.* **175**, 1159–1168 https://doi.org/10.1086/520354
108 Hoppe, H.C., de Wet, B.J., Cywes, C., Daffe, M. and Ehlers, M.R. (1997) Identification of phosphatidylinositol mannoside as a mycobacterial adhesin mediating both direct and opsonic binding to nonphagocytic mammalian cells. *Infect. Immun.* **65**, 3896–3905 PMID:9284169
109 Selvaraj, P., Jawahar, M.S., Rajeswari, D.N., Alagarasu, K., Vidyarani, M. and Narayanan, P.R. (2006) Role of mannose binding lectin gene variants on its protein levels and macrophage phagocytosis with live *Mycobacterium tuberculosis* in pulmonary tuberculosis. *FEMS Immunol. Med. Microbiol.* **46**, 433–437 https://doi.org/10.1111/j.1574-695X.2006.00053.x
110 Joller, N., Weber, S.S., Muller, A.J., Sporri, R., Selchow, P., Sander, P. et al. (2010) Antibodies protect against intracellular bacteria by Fc receptor-mediated lysosomal targeting. *Proc. Natl Acad. Sci. U.S.A.* **107**, 20441–20446 https://doi.org/10.1073/pnas.1013827107
111 Chen, T., Blanc, C., Eder, A.Z., Prados-Rosales, R., Souza, A.C., Kim, R.S. et al. (2016) Association of human antibodies to arabinomannan with enhanced mycobacterial opsonophagocytosis and intracellular growth reduction. *J. Infect. Dis.* **214**, 300–310 https://doi.org/10.1093/infdis/jiw141
112 Prados-Rosales, R., Carreno, L., Cheng, T., Blanc, C., Weinrick, B., Malek, A. et al. (2017) Enhanced control of *Mycobacterium tuberculosis* extrapulmonary dissemination in mice by an arabinomannan-protein conjugate vaccine. *PLoS Pathog.* **13**, e1006250 https://doi.org/10.1371/journal.ppat.1006250
113 Tailleux, L., Schwartz, O., Herrmann, J.L., Pivert, E., Jackson, M., Amara, A. et al. (2003) DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med.* **197**, 121–127 https://doi.org/10.1084/jem.20021468
114 Geurtsen, J., Chedammi, S., Mesters, J., Cot, M., Driessen, N.N., Sambou, T. et al. (2009) Identification of mycobacterial α-glucan as a novel ligand for DC-SIGN: involvement of mycobacterial capsular polysaccharides in host immune modulation. *J. Immunol.* **183**, 5221–5231 https://doi.org/10.4049/jimmunol.0900768
115 Ehlers, S. (2010) DC-SIGN and mannosylated surface structures of *Mycobacterium tuberculosis*: a deceptive liaison. *Eur. J. Cell Biol.* **89**, 95–101 https://doi.org/10.1016/j.ejcb.2009.10.004
116 Hickey, T.B., Thorson, L.M., Speert, D.P., Daffe, M. and Stokes, R.W. (2009) *Mycobacterium tuberculosis* Cpn60.2 and DnaK are located on the bacterial surface, where Cpn60.2 facilitates efficient bacterial association with macrophages. *Infect. Immun.* **77**, 3389–3401 https://doi.org/10.1128/IAI.00143-09
117 Hickey, T.B., Ziltener, H.J., Speert, D.P. and Stokes, R.W. (2010) *Mycobacterium tuberculosis* employs Cpn60.2 as an adhesin that binds CD43 on the macrophage surface. *Cell Microbiol.* **12**, 1634–1647 https://doi.org/10.1111/j.1462-5822.2010.01496.x
118 Diaz-Silvestre, H., Espinosa-Cueto, P., Sanchez-Gonzalez, A., Esparza-Ceron, M.A., Pereira-Suarez, A.L., Bernal-Fernandez, G. et al. (2005) The 19-kDa antigen of *Mycobacterium tuberculosis* is a major adhesin that binds the mannose receptor of THP-1 monocytic cells and promotes phagocytosis of mycobacteria. *Microb. Pathog.* **39**, 97–107 https://doi.org/10.1016/j.micpath.2005.06.002
119 Hetland, G. and Wiker, H.G. (1994) Antigen 85C on *Mycobacterium bovis*, BCG and *M. tuberculosis* promotes monocyte-CR3-mediated uptake of microbeads coated with mycobacterial products. *Immunology* **82**, 445–449 PMID:7959881
120 Mueller-Ortiz, S.L., Wanger, A.R. and Norris, S.J. (2001) Mycobacterial protein HbhA binds human complement component C3. *Infect. Immun.* **69**, 7501–7511 https://doi.org/10.1128/IAI.69.12.7501-7511.2001
121 Mueller-Ortiz, S.L., Sepulveda, E., Olsen, M.R., Jagannath, C., Wanger, A.R. and Norris, S.J. (2002) Decreased infectivity despite unaltered C3 binding by a DeltahbhA mutant of *Mycobacterium tuberculosis*. *Infect. Immun.* **70**, 6751–6760 https://doi.org/10.1128/IAI.70.12.6751-6760.2002
122 Villeneuve, C., Gilleron, M., Maridonneau-Parini, I., Daffe, M., Astarie-Dequeker, C. and Etienne, G. (2005) Mycobacteria use their surface-exposed glycolipids to infect human macrophages through a receptor-dependent process. *J. Lipid Res.* **46**, 475–483 https://doi.org/10.1194/jlr.M400308-JLR200
123 Torrelles, J.B., Azad, A.K. and Schlesinger, L.S. (2006) Fine discrimination in the recognition of individual species of phosphatidyl-myo-inositol mannosides from *Mycobacterium tuberculosis* by C-type lectin pattern recognition receptors. *J. Immunol.* **177**, 1805–1816 https://doi.org/10.4049/jimmunol.177.3.1805
124 Gagliardi, M.C., Lemassu, A., Teloni, R., Mariotti, S., Sargentini, V., Pardini, M. et al. (2007) Cell wall-associated α-glucan is instrumental for *Mycobacterium tuberculosis* to block CD1 molecule expression and disable the function of dendritic cell derived from infected monocyte. *Cell Microbiol.* **9**, 2081–2092 https://doi.org/10.1111/j.1462-5822.2007.00940.x
125 Ellner, J.J. and Daniel, T.M. (1979) Immunosuppression by mycobacterial arabinomannan. *Clin. Exp. Immunol.* **35**, 250–257 PMID:108041

126 Horii, T., Yoshinaga, K., Kobayashi, N., Seto, K., Orikawa, Y., Okamoto, M. et al. (2014) Z-100, an immunomodulatory extract of *Mycobacterium tuberculosis* strain Aoyama B, prevents spontaneous lymphatic metastasis of B16-BL6 melanoma. *Biol. Pharm. Bull.* **37**, 642–647 https://doi.org/10.1248/bpb.b13-00927
127 Tanne, A., Ma, B., Boudou, F., Tailleux, L., Botella, H., Badell, E. et al. (2009) A murine DC-SIGN homologue contributes to early host defense against *Mycobacterium tuberculosis*. *J. Exp. Med.* **206**, 2205–2220 https://doi.org/10.1084/jem.20090188
128 Geijtenbeek, T.B., Van Vliet, S.J., Koppel, E.A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C.M., Appelmelk, B. et al. (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* **197**, 7–17 https://doi.org/10.1084/jem.20021229
129 Mazurek, J., Ignatowicz, L., Kallenius, G., Svenson, S.B., Pawlowski, A. and Hamasur, B. (2012) Divergent effects of mycobacterial cell wall glycolipids on maturation and function of human monocyte-derived dendritic cells. *PLoS One* **7**, e42515 https://doi.org/10.1371/journal.pone.0042515
130 Jozefowski, S., Sobota, A., Pawlowski, A. and Kwiatkowska, K. (2011) *Mycobacterium tuberculosis* lipoarabinomannan enhances LPS-induced TNF-α production and inhibits NO secretion by engaging scavenger receptors. *Microb. Pathog.* **50**, 350–359 https://doi.org/10.1016/j.micpath.2011.03.001
131 Yonekawa, A., Saijo, S., Hoshino, Y., Miyake, Y., Ishikawa, E., Suzukawa, M. et al. (2014) Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. *Immunity* **41**, 402–413 https://doi.org/10.1016/j.immuni.2014.08.005
132 van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J. et al. (2007) *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* **129**, 1287–1298 https://doi.org/10.1016/j.cell.2007.05.059
133 Ciaramella, A., Cavone, A., Santucci, M.B., Garg, S.K., Sanarico, N., Bocchino, M. et al. (2004) Induction of apoptosis and release of interleukin-1 β by cell wall-associated 19-kDa lipoprotein during the course of mycobacterial infection. *J. Infect. Dis.* **190**, 1167–1176 https://doi.org/10.1086/423850
134 Harding, C.V. and Boom, W.H. (2010) Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nat. Rev. Microbiol.* **8**, 296–307 https://doi.org/10.1038/nrmicro2321
135 Georgieva, M., Sia, J.K., Bizzell, E., Madan-Lala, R. and Rengarajan, J. (2018) *Mycobacterium tuberculosis* GroEL2 modulates dendritic cell responses. *Infect. Immun.* **86**, e00387-17 https://doi.org/10.1128/IAI.00387-17
136 Rousseau, C., Winter, N., Pivert, E., Bordat, Y., Neyrolles, O., Ave, P. et al. (2004) Production of phthiocerol dimycocerosates protects *Mycobacterium tuberculosis* from the cidal activity of reactive nitrogen intermediates produced by macrophages and modulates the early immune response to infection. *Cell Microbiol.* **6**, 277–287 https://doi.org/10.1046/j.1462-5822.2004.00368.x
137 Cambier, C.J., Takaki, K.K., Larson, R.P., Hernandez, R.E., Tobin, D.M., Urdahl, K.B. et al. (2014) Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. *Nature* **505**, 218–222 https://doi.org/10.1038/nature12799
138 Aguilo, J.I., Alonso, H., Uranga, S., Marinova, D., Arbues, A., de Martino, A. et al. (2013) ESX-1-induced apoptosis is involved in cell-to-cell spread of *Mycobacterium tuberculosis*. *Cell Microbiol.* **15**, 1994–2005 https://doi.org/10.1111/cmi.12169
139 Gilleron, M., Quesniaux, V.F. and Puzo, G. (2003) Acylation state of the phosphatidylinositol hexamannosides from *Mycobacterium bovis* bacillus Calmette Guerin and *Mycobacterium tuberculosis* H37Rv and its implication in Toll-like receptor response. *J Biol Chem.* **278**, 29880–29889 https://doi.org/10.1074/jbc.M303446200
140 Bansal, K., Narayana, Y. and Balaji, K.N. (2009) Inhibition of TNF-α-induced cyclooxygenase-2 expression by *Mycobacterium bovis* BCG in human alveolar epithelial A549 cells. *Scand. J. Immunol.* **69**, 11–19 https://doi.org/10.1111/j.1365-3083.2008.02190.x
141 Toyonaga, K., Torigoe, S., Motomura, Y., Kamichi, T., Hayashi, J.M., Morita, Y.S. et al. (2016) C-Type lectin receptor DCAR recognizes mycobacterial phosphatidyl-inositol mannosides to promote a Th1 response during infection. *Immunity* **45**, 1245–1257 https://doi.org/10.1016/j.immuni.2016.10.012
142 Takala, A.K., Eskola, J. and van Alphen, L. (1990) Spectrum of invasive *Haemophilus influenzae* type b disease in adults. *Arch. Intern. Med.* **150**, 2573–2576 https://doi.org/10.1001/archinte.1990.00390230113016
143 Jann, K. and Jann, B. (1990) *Bacterial Capsules*, Springer, Berlin, Heidelberg, New York
144 Torrelles, J.B., Knaup, R., Kolareth, A., Slepushkina, T., Kaufman, T.M., Kang, P. et al. (2008) Identification of *Mycobacterium tuberculosis* clinical isolates with altered phagocytosis by human macrophages due to a truncated lipoarabinomannan. *J. Biol. Chem.* **283**, 31417–31428 https://doi.org/10.1074/jbc.M806350200
145 Khoo, K.H., Douglas, E., Azadi, P., Inamine, J.M., Besra, G.S., Mikusova, K. et al. (1996) Truncated structural variants of lipoarabinomannan in ethambutol drug-resistant strains of *Mycobacterium smegmatis*. Inhibition of arabinan biosynthesis by ethambutol. *J. Biol. Chem.* **271**, 28682–28690 https://doi.org/10.1074/jbc.271.45.28682
146 Birch, H.L., Alderwick, L.J., Appelmelk, B.J., Maaskant, J., Bhatt, A., Singh, A. et al. (2010) A truncated lipoglycan from mycobacteria with altered immunological properties. *Proc. Natl Acad. Sci. U.S.A.* **107**, 2634–2639 https://doi.org/10.1073/pnas.0915082107
147 Korkegian, A., Roberts, D.M., Blair, R. and Parish, T. (2014) Mutations in the essential arabinosyltransferase EmbC lead to alterations in *Mycobacterium tuberculosis* lipoarabinomannan. *J. Biol. Chem.* **289**, 35172–35181 https://doi.org/10.1074/jbc.M114.583112
148 van de Weerd, R., Boot, M., Maaskant, J., Sparrius, M., Verboom, T., van Leeuwen, L.M. et al. (2016) Inorganic phosphate limitation modulates capsular polysaccharide composition in mycobacteria. *J. Biol. Chem.* **291**, 11787–11799 https://doi.org/10.1074/jbc.M116.722454
149 Ates, L.S., van der Woude, A.D., Bestebroer, J., van Stempvoort, G., Musters, R.J., Garcia-Vallejo, J.J. et al. (2016) The ESX-5 system of pathogenic mycobacteria is involved in capsule integrity and virulence through its substrate PPE10. *PLoS Pathog.* **12**, e1005696 https://doi.org/10.1371/journal.ppat.1005696
150 Asencion Diez, M.D., Demonte, A.M., Syson, K., Arias, D.G., Gorelik, A., Guerrero, S.A. et al. (2015) Allosteric regulation of the partitioning of glucose-1-phosphate between glycogen and trehalose biosynthesis in *Mycobacterium tuberculosis*. *Biochim. Biophys. Acta* **1850**, 13–21 https://doi.org/10.1016/j.bbagen.2014.09.023
151 Leiba, J., Syson, K., Baronian, G., Zanella-Cleon, I., Kalscheuer, R., Kremer, L. et al. (2013) *Mycobacterium tuberculosis* maltosyltransferase GlgE, a genetically validated antituberculosis target, is negatively regulated by Ser/Thr phosphorylation. *J. Biol. Chem.* **288**, 16546–16556 https://doi.org/10.1074/jbc.M112.398503
152 Pollard, A.J., Perrett, K.P. and Beverley, P.C. (2009) Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. *Nat. Rev. Immunol.* **9**, 213–220 https://doi.org/10.1038/nri2494
153 Macleod, C.M., Hodges, R.G., Heidelberger, M. and Bernhard, W.G. (1945) Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. *J. Exp. Med.* **82**, 445–465 https://doi.org/10.1084/jem.82.6.445

Biochemical Journal (2019) **476** 1995–2016
https://doi.org/10.1042/BCJ20190324

154 Lienhardt, C., Kraigsley, A.M. and Sizemore, C.F. (2016) Driving the way to tuberculosis elimination: the essential role of fundamental research. *Clin. Infect. Dis.* **63**, 370–375 https://doi.org/10.1093/cid/ciw250

155 Hamasur, B., Haile, M., Pawlowski, A., Schroder, U., Williams, A., Hatch, G. et al. (2003) *Mycobacterium tuberculosis* arabinomannan-protein conjugates protect against tuberculosis. *Vaccine* **21**, 4081–4093 https://doi.org/10.1016/S0264-410X(03)00274-3

156 Haile, M., Hamasur, B., Jaxmar, T., Gavier-Widen, D., Chambers, M.A., Sanchez, B. et al. (2005) Nasal boost with adjuvanted heat-killed BCG or arabinomannan-protein conjugate improves primary BCG-induced protection in C57BL/6 mice. *Tuberculosis (Edinb)* **85**, 107–114 https://doi.org/10.1016/j.tube.2004.09.013

157 Teitelbaum, R., Glatman-Freedman, A., Chen, B., Robbins, J.B., Unanue, E., Casadevall, A. et al. (1998) A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. *Proc. Natl Acad. Sci. U.S.A.* **95**, 15688–15693 https://doi.org/10.1073/pnas.95.26.15688

158 Li, H., Wang, X.X., Wang, B., Fu, L., Liu, G., Lu, Y. et al. (2017) Latently and uninfected healthcare workers exposed to TB make protective antibodies against *Mycobacterium tuberculosis*. *Proc. Natl Acad. Sci. U.S.A.* **114**, 5023–5028 https://doi.org/10.1073/pnas.1611776114

159 Guerin, M.E., Kordulakova, J., Alzari, P.M., Brennan, P.J. and Jackson, M. (2010) Molecular basis of phosphatidyl-myo-inositol mannoside biosynthesis and regulation in mycobacteria. *J. Biol. Chem.* **285**, 33577–33583 https://doi.org/10.1074/jbc.R110.168328