Mycobacterium tuberculosis adhesins: potential biomarkers as anti-tuberculosis therapeutic and diagnostic targets

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Introduction

Mycobacterium tuberculosis, the aetiological agent of tuberculosis (TB), continues to pose a challenge to global public health. Despite more than a century of research, this non-discriminant pathogen continues to infect roughly one-third of the world’s population (WHO, 2013). Whilst there has been a significant reduction in TB cases and deaths in the past two decades, approximately 8.6 million new cases and 1.3 million people succumbed to the disease in 2012, despite the availability of cheap, efficacious and curative therapy for TB (WHO, 2013). The synergistic relationship between human immunodeficiency virus (HIV) and M. tuberculosis infection, and the significant increase in the prevalence of multi-, extensively and totally drug-resistant M. tuberculosis strains (Gillespie, 2002; Fauci et al., 2008; Jassal & Bishai, 2009; LoBue, 2009; Velayati et al., 2009; Almeida Da Silva & Palomino, 2011) are largely accountable for the dramatic resurgence of TB as a serious global public health epidemic. This is further complicated by the lack of an effective vaccine (Russell et al., 2010), prolonged chemotherapy regimens (Mitchion & Davies, 2012) and adverse TB/HIV drug interactions (Luetkemeyer et al., 2011). Knowledge on the mechanisms utilized by M. tuberculosis to infect the host would offer novel perspective and define new targets to facilitate the design and development of drugs that are effective against both sensitive and resistant organisms (Ginsberg & Spigelman, 2007), efficacious vaccines (Bermudez et al., 2002), as well as crucially needed rapid, accurate and cheap point-of-care tests (Wallis et al., 2010).

It is well established that adherence molecules (adhesins) play a fundamental role in the pathogen–host interaction (da Silva Neto et al., 2009; Espitia et al., 2012). Invasion of host cells by bacteria is a complex process involving both bacterial and host cell determinants (Bermudez & Goodman, 1996; Danelishvili et al., 2003). Pathogenic bacteria, such as M. tuberculosis, must initially adhere to and invade eukaryotic cells as a survival mechanism, enabling host colonization and the evasion of host immune defences (Niemann et al., 2004; Pizarro-Cerdá & Cossart, 2006; Kline et al., 2009). Adherence and invasion mechanisms have been well studied in pathogenic bacteria and fungi (Finlay & Cossart, 1997; Pizarro-Cerdá & Cossart, 2006; Singh et al., 2012a; Monack & Hultgren, 2013; Foster et al., 2014). These have illustrated that adhesins are the key players in the interactions that occur between the pathogen and the host, operating either as intercellular adhesion molecules or substrate adhesion molecules (da Silva Neto et al., 2009). In this capacity, they are able to facilitate either cell-to-cell or cell-to-extracellular matrix (ECM) adherence and are usually surface-exposed (da Silva Neto et al., 2009). Adhesins also function in surface colonization and bacterial cell aggregation by facilitating cell-to-cell contact, leading to the formation of microbial community structures or biofilms, a key contributor to microbial persistence (Barnhart & Chapman, 2006). Adhesins are therefore essential to microbial pathogenesis and are considered important biomarkers for diagnostics and therapeutics.

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Abbreviations: Apa, alanine-proline-rich antigen; BCG, Bacille Calmette–Guérin; ECM, extracellular matrix; GAPDH, glyceraldehyde–3-phosphate-dehydrogenase; GBS, group B Streptococcus; HBHA, heparin-binding haemagglutinin; HIV, human immunodeficiency virus; MTP, M. tuberculosis pil; PSP-A, pulmonary surfactant protein-A; TB, tuberculosis.
The initial interaction of the bacilli with the host immune system has been reported to occur in the lungs with the alveolar macrophage, the host cell within which *M. tuberculosis* replicates (Algood et al., 2003; Smith, 2003). Under optimal conditions, these immune cells engulf the bacterium into a phagosome, leading to its destruction (Dubnau & Smith, 2003). However, *M. tuberculosis* has evolved mechanisms that allow it to thrive within the harsh environment, by delaying phagosome maturation (Armstrong & Hart, 1971; Russell, 2001; Nguyen & Pieters, 2005). If this fails, the bacteria are able to escape death by entering the cytosol (van der Wel et al., 2007). TB-infected macrophages can travel to the hilar lymph nodes and bloodstream (Henderson et al., 1963; Harmsen et al., 1985) and this interaction is critical in the dissemination and systematic spread of the pathogen.

Although the primary mechanism of infection is reported to be via macrophages, epithelial cells are present in far larger numbers than macrophages within alveoli (Crandall & Kim, 1991). The first cells that *M. tuberculosis* encounters in the lung are therefore most likely to be epithelial cells. *M. tuberculosis* is versatile in that it is capable of infecting and growing in these pneumocytes *ex vivo* (Bermudez & Goodman, 1996; Mehta et al., 1996; Ashiru et al., 2010). The *M. tuberculosis*–epithelial cell interaction may potentially precede invasion of the macrophage and could also then facilitate the recruitment of macrophages to the site of infection by chemokine secretion (Alteri, 2005). Epithelial cells also present a niche within which the bacilli are afforded direct access to the host lymphatic and blood systems without the need for carrier macrophages. This is affected by the disruption and destruction of the alveolar vascular endothelium due to the cytotoxicity of *M. tuberculosis* to human pneumocytes (McDonough & Kress, 1995; Dobos et al., 2000; Castro-Garza et al., 2002).

Dendritic cells are better antigen presenters than macrophages and are key players in the early stages of TB infection (Tascon et al., 2000) and activate T cells with specific *M. tuberculosis* antigens (Bodnar et al., 2001; Gonzalez-Juarrero & Orme, 2001). Dendritic cells, however, do not support intracellular growth of *M. tuberculosis* but maintain the live bacteria in vacuoles (Tailleux et al., 2003). As dendritic cells are migratory, they may potentially facilitate the dissemination of *M. tuberculosis* in this way (Lipscomb & Masten, 2002).

Microbial adhesins are crucial to bacterial attachment to host cells (Fig. 1). The interaction of bacterial adhesins with host cell receptors is a key determinant of host specificity and tissue tropism (Klemm & Schembri, 2000). Furthermore, the cell surface location of adhesins and their role in attachment to host cells leads to the triggering of immune responses, which are crucial to the host’s defence against infection (Barnhart & Chapman, 2006; Bergsten et al., 2007).

**Bacterial adhesins and their mechanisms of interaction with the host**

Adhesins serve to fulfill a common basic role, which is to initiate close contact with a receptor-like domain on the host cell surface, forming a fundamental link between the host and bacterium (Gerlach & Hensel, 2007). Infectious organisms have evolved to express novel adhesins that are able to bind to molecules ordinarily located on the eukaryotic cell surface. Molecules such as integrins, glycosaminoglycans and specific sugar residues have all been shown to interact with adhesins of pathogens (Brennan et al., 2001; Delogu & Brennan, 1999; Dersch & Isberg, 2000). Most bacteria express a number of variable adhesins on their surfaces that have specific affinity to terminal sugar residues or internal sequences in oligosaccharide chains that help to define the microbe’s ecological niche (Esco & Sharon, 2009). A range of adhesins have been described and characterized. Lectin adhesins function
predominantly to interact with glycan ligand receptors on the surface of host cells (Esko & Sharon, 2009). In addition, two major classes of protein adhesins have been defined to date: the fimbrial and the non-fimbrial adhesins (Gerlach & Hensel, 2007). Fimbriae (hairs) or pili (threads) are the most common form of bacterial adhesins. They are elongated, multi-subunit protein structures that are able to interact with glycoprotein and glycolipid receptors found on host cells (Esko & Sharon, 2009).

To be functional, adhesins must be anchored onto or displayed on the bacterial cell surface with their functional domain on display (Chhatwal, 2002). Bacteria have developed several anchoring mechanisms involving the presence of a signal peptide or unique motifs (Chhatwal, 2002). The majority of adhesins function by binding ECM components, such as fibronectin. This is often considered to be an essential binding molecule facilitating bacterial adherence, due largely to its ability to bind both host cells and bacteria (Henderson et al., 2011). Fibronectin is also regarded as an effector in the triggering of signal transduction events that result in bacterial invasion of eukaryotic cells through interaction with integrins (Ioh et al., 1999; Chhatwal, 2002). M. tuberculosis, specifically, has been shown to express adhesins that are capable of binding to ECM proteins, such as proteoglycans and fibronectin (Brennan et al., 2001).

Proteins commonly secreted by bacterial pathogens can, in some instances, be ‘anchorless adhesins’ that facilitate colonization of host organisms (Gerlach & Hensel, 2007). Following their secretion, they accumulate and reassociate with the bacterial surface where they are able to execute biological functions including host adherence and entry (Bergmann et al., 2001; Chhatwal, 2002).

**Adhesins central to M. tuberculosis pathogenesis**

Several studies have identified multiple M. tuberculosis proteins capable of interacting with receptors on host cells to facilitate binding to mammalian components and these are classified as mycobacterial adhesins (Fig. 2) (Menozzi et al., 2006; Kumar et al., 2013). Pethe et al. (2002) identified a laminin binding protein involved in cyto-adherence by its recognition of laminin. Kinhikar et al. (2006) showed that the glyoxalate pathway enzyme, malate synthase (glcB; Rv1837c), binds to the human ECM proteins laminin and fibronectin and is an anchorless adhesin. The 19 kDa lipoprotein antigen (Rv3763) present on the cell wall preferentially binds to THP-1 macrophage-like cells (Diaz-Silvestre et al., 2005). The cell surface glycoprotein alanine-proline-rich antigen (Apa, Rv1860), initially considered a secreted molecule, has been shown to transiently associate with the cell wall to allow attachment to the pulmonary surfactant protein-A (PSP-A) (Ragas et al., 2007). The Cpn60.2 molecular chaperone protein (GroEL2; Rv0440), believed to be involved in bacterial pathogenicity and considered essential for cell viability, appears to be necessary to facilitate efficient bacterial association with macrophages

**Heparin-binding haemagglutinin adhesin (hbhA; Rv0475)**

The most characterized and major adhesin in M. tuberculosis is the 28 kDa heparin-binding haemagglutinin adhesin (HBHA). This surface-exposed protein is a virulence factor that facilitates the dissemination of M. tuberculosis from the site of primary infection by initiating interaction with host epithelial cells (Menozzi et al., 1996, 1998, 2006; Pethe et al., 2001; Esposito et al., 2011). The two crucial steps in TB pathogenesis, namely bacterial aggregation and cell adhesion, are facilitated by HBHA (Menozzi et al., 1998; Esposito et al., 2012). HBHA-mediated aggregation is instrumental in the formation of bacterial clumps, allowing for more effective adherence and invasion (Esposito et al., 2012; Lebrun et al., 2012). A definitive role for HBHA in the facilitation of host adherence was shown by Menozzi et al. (1996), in which antibodies directed against HBHA inhibited attachment of mycobacteria to epithelial cells. In support of this, patients with active TB have been shown to produce anti-HBHA antibodies, suggesting HBHA expression during human infection (Menozzi et al., 1998). The carboxy-terminal lysine-rich domain of HBHA functions
in recognizing heparan sulphate-containing receptors on epithelial cells (Delogu & Brennan, 1999; Pethe et al., 2000). Pethe et al. (2001) showed that colonization of an M. tuberculosis hbbA mutant strain in the lungs of mice was equivalent to that of the wild-type strain. However, the mutant displayed a reduced capacity to disseminate from the lungs to other regions of the body, suggesting the role of HBHA in extrapulmonary spread. The authors also showed that an antibody against the carboxyl-terminal domain of HBHA blocks binding to epithelial cell receptors, impeding extrapulmonary spread of M. tuberculosis in the mouse model (Pethe et al., 2001). This suggests that the humoral immune response to HBHA, and possibly other M. tuberculosis adhesins, could potentially play a protective role in blocking dissemination from the lungs (Alteri, 2005).

**M. tuberculosis pili**

Pili proteins are hydrophobic adhesion molecules used by a wide range of bacterial pathogens to infect host cells (Finlay & Falkow, 1997). Structurally, pili are generally composed of pilin subunits with an adhesin tip, fashioned into straight or flexible filaments 1–10 nm wide and 0.07–3 μm long (Telford et al., 2006). Several virulence-associated functions, including agglutination of human and animal erythrocytes, bacterial adherence/aggregation, biofilm formation, and adherence and colonization of mucosal surfaces can be functionally attributed to pili (Strom & Lory, 1993; Finlay & Falkow, 1997). The hydrophobicity of the pilin adhesin enables interaction between bacteria and eukaryotic cells (Klemm & Schembri, 2000). Several distinct pilus types have been identified, the most characterized of which are the type I pili (produced by enteropathogenic *Escherichia coli*), type IV pili (produced by *E. coli* and *Pseudomonas* and *Neisseria* species) and curli pili (produced by some strains of *E. coli*) (Telford et al., 2006). The previous misconception that mycobacteria are not piliated was clarified by Alteri (2005), who proved, using transmission electron microscopy of negatively stained bacilli, that *M. tuberculosis* produces two physically distinct pili morphotypes: type IV and curli-like pili (MTP).

The type IVB pilus locus of *M. tuberculosis* encodes a prepilin of the Flp pili family (Alteri, 2005). In addition, the proteins encoded by the Rv0990c and Rv2551c ORFs are thought to be involved in the secretion or cleavage of the Flp prepilin substrate (Alteri, 2005). Genetic analysis revealed that *M. tuberculosis* acquired these genes by horizontal gene transfer as the *flp* genes were shown to be flanked by multiple direct repeats, suggesting insertion of foreign DNA into its chromosome (Alteri, 2005). The role of this pilus type as an adhesin in this organism has yet to be determined. In Gram-negative organisms, type IV pilin function in adherence to host tissues, co-aggregation, immunomodulation, motility and DNA uptake (Telford et al., 2006).

Alteri et al. (2007) showed that purified MTP comprise 4 kDa protein subunits, encoded by the Rv3312A ORF. These researchers also demonstrated that MTP are produced during pathogenesis and play a role in stimulating the humoral immune response. This was evidenced by the sera of patients with active TB containing IgG antibodies against MTP (Alteri et al., 2007). Furthermore, MTP bind to laminin *in vitro* and are produced during adherence to epithelial cells, implying that they serve as an adherence factor, crucial in mediating close interaction and colonization with host cells (Alteri et al., 2007). Their role as an adherence factor was further substantiated by their involvement in cellular aggregation and biofilm formation (Ramsugit et al., 2013) and in the adhesion to, and invasion of, THP-1 macrophages (Ramsugit & Pillay, in press).

**Apa (alanine-proline-rich antigen; Rv1860)**

The 45–47 kDa secretory and cell surface antigen Apa is a mycobacterial glycoprotein whose expression appears to be restricted to members of the *M. tuberculosis* complex, including the vaccine strain *M. bovis* Bacille Calmette–Guérin (BCG) (Ragas et al., 2007; Nandakumar et al., 2013). The secreted antigen is not present in other mycobacterial species, including *M. avium*, *M. marinum* or *M. smegmatis* (Ragas et al., 2007; Nandakumar et al., 2013). Apa is targeted by, and binds directly to, the human PSP-A, an innate immune system C-type lectin responsible for early recognition of invading pathogens (Ragas et al., 2007). The secreted antigen remains associated with the cell wall only long enough to facilitate its attachment to PSP-A. Ragas et al. (2007) demonstrated the presence of a structural determinant in the manno-oligosaccharide moiety that has been highly implicated in the adhesion function of the protein. They also confirmed the adhesion function of Apa during TB infection and pathogenesis. The immune-dominant Apa possesses fibronectin-binding activity and is strongly recognized by serum antibodies of active TB patients. In addition, it shares significant amino acid homology with a fibronectin attachment protein family common to other mycobacterial species, including *M. avium*, *M. marinum* and *M. leprae* (Nandakumar et al., 2013). In *M. tuberculosis*, specifically, mannosylated Apa plays a key role in host cell interaction (Nandakumar et al., 2013). Apa has been proposed as a possible vaccine candidate or component for future vaccines against TB. Nandakumar et al. (2013) showed that Apa offered significant protection against virulent *M. tuberculosis* in mice when used as a BCG-booster vaccine.

**Malate synthase (glcB; Rv1837c)**

The glyoxalate shunt, a key pathway in fatty acid metabolism of *M. tuberculosis* during persistent infection, has long been hypothesized to be a weakness in the bacteria’s armour that could potentially be exploited for the development of anti-tubercular therapeutics (Krieger et al., 2012). da Silva Neto et al. (2009) showed that malate...
synthase, an enzyme of the glyoxalate pathway, from *Paracoccidioides brasiliensis* is a multifunctional protein, with a dual role as an enzyme and mediates adherence of the fungus to host cells via its ability to bind fibronectin and type I/type IV collagen. Malate synthase has been suggested to be actively secreted by this fungal pathogen in a similar manner to *M. tuberculosis* (da Silva Neto et al., 2009). In *M. tuberculosis*, the single malate synthase encoded by *glcB* is thought to play a key role in pathogenesis by imparting some degree of virulence to the bacteria (Dunn et al., 2009). Kinhikar et al. (2006) have demonstrated in vivo expression of malate synthase to be typical during active infection, with the enzymic function suggesting a general cytoplasmic localization of the protein. However, this protein was also consistently identified as a secreted protein in the growth medium filtrates of mid-exponential phase cultures (Kinhikar et al., 2006). Studies have shown that secreted malate synthase enhances adherence of the pathogen to lung epithelial cells by binding to the glycoproteins, laminin and fibronectin (Kinhikar et al., 2006; Dunn et al., 2009). The binding activity of malate synthase was shown to be mediated by the presence of a unique C-terminal domain. These features, together with the marked absence of a conventional secretion mechanism or defined cell-wall-anchoring motif, resulted in the malate synthase of *M. tuberculosis* being classified as an anchorless adhesin that is able to contribute to bacterial virulence by facilitating infection of the host and dissemination (Kinhikar et al., 2006).

**Glyceraldehyde-3-phosphate dehydrogenase (gap; Rv1436)**

The GAPDH protein family is known to have diverse functional activity depending on the subcellular location, secondary to its primary role in glycolysis and central carbon metabolism (Barbosa et al., 2006). Several authors have indicated that GAPDH may be either secreted or expressed on the cell surface in both Gram-positive and Gram-negative bacteria, parasites, and fungi (Pancholi, 2001; Pancholi & Chhatwal, 2003; Matta et al., 2010; Jin et al., 2011). In support of this, the GAPDH of group A streptococci has been shown to be membrane-bound and is able to bind fibronectin, lysozyme, and myosin and actin cytoskeletal proteins, pointing toward an additional function leading to bacterial colonization (Barbosa et al., 2006). The unusual extracellular localization of GAPDH has been confirmed in a broad range of other microorganisms, including *Staphylococcus* species, *Neisseria meningitidis*, *E. coli*, *P. brasiliensis*, *Trichomonas vaginalis* and *Candida albicans* (Dumke et al., 2011).

The surface-localized equivalent proteins have also been shown to remain enzymically active and are transcribed from the same ORF as the cytoplasmic molecules (Purves et al., 2010). In its role as a surface-associated protein, GAPDH is able to interact with both specified host factors like fibronectin, fibrinogen, albumin, laminin, collagen and plasminogen, as well as human epithelial and endothelial cells and fimbriae of other bacterial species (Dumke et al., 2011). GAPDH, specifically, has been shown to be important in the pathogenesis of *Staphylococcus aureus* infections (Purves et al., 2010). Tunio et al. (2010) showed an *N. meningitidis* gapA mutant to adhere significantly less to human cells when compared with wild-type strains. Additionally, Boël et al. (2005) indicated that inhibiting export of GAPDH to the surface of group A streptococci affected its virulence by reducing adherence to target cells.

Despite the existence of a large body of literature on bacterial cell surface GAPDH, only one study has shown direct evidence for the role of GAPDH as a cell surface protein playing a role in bacterial virulence (Henderson & Martin, 2011). This is probably attributed to the essentiality of the gene in its primary function in glycolysis, resulting in the inability to inactivate the encoding gene (Henderson & Martin, 2011). It is thus not surprising that a cell surface GAPDH knockout has, to date, not been evaluated in vivo.

**Chaperonins as adhesins (dnaK and groEL2; Rv0350 and Rv0440)**

Although a number of heat-shock protein classes have been identified, GroESL and DnaK are regarded as the major response systems within bacteria (Singh et al., 2012b). The molecular chaperones constitute a diverse set of conserved proteins in bacteria, encoded by the essential heat-shock protein genes *groEL* and *groES*. These genes, also called *cpn60* and *cpn10*, mediate the correct assembly, folding, transport and degradation of other proteins in vivo (Qamra et al., 2004; Shahar et al., 2011; Singh et al., 2012b). *M. tuberculosis* contains two copies of the *cpn60* genes, with one of these genes, *cpn60.1*, organized in an operon with *cpn10*, while the second, *cpn60.2*, is arranged separately in the genome and is expressed from the *myc28* gene (Qamra et al., 2004; Lewthwaite et al., 2007; Shahar et al., 2011).

Recent mycobacterial studies demonstrated the unusual presence of the Cpn60s on the outside surface (capsule) of the bacterial cell. This is despite the predominant role of these proteins in the cytoplasm and the lack of a secretion signal sequence or other known motif implicating its export (Qamra et al., 2004; Shahar et al., 2011; Zhu et al., 2013). Hickey et al. (2009) have shown both the molecular chaperones Cpn60.2 and DnaK to be surface components of the mycobacterial capsule in cultured bacilli. The authors also showed Cpn60.2 to bind to the surface of macrophages at sites required for efficient association with *M. tuberculosis* bacilli, and that blocking surface-localized Cpn60.2 with antibodies resulted in a reduction of bacterial binding. Additionally, Hickey et al. (2009) showed that the Cpn60.2 protein is a major mycobacterial adhesin, able to stabilize the interaction between the pathogen and the alveolar macrophage through CD43. Such studies implicate Cpn60.2 as having a secondary function as an adhesin molecule (Hickey et al., 2010; Shahar et al., 2011). They provided
further evidence that mycobacterial molecular chaperone cell stress molecules are vital moonlighting proteins, able to promote bacterial survival and virulence due to their irregular position outside the cell (Henderson et al., 2010). It was hypothesized that the secondary, extracellular functionality of these molecular chaperones may allow for synergism with the intracellular cell stress response to generate a novel homeostatic network of interactions (Henderson et al., 2010). Although the Cpn60.2 protein is expressed from outside the GroEL/ES-like operon, studies have shown the protein to be essential for survival, with deletion of the genes encoding Cpn60.2 completely preventing the growth of M. tuberculosis (Shahar et al., 2011). Hu et al. (2008) demonstrated an inability to produce M. tuberculosis mutants lacking the cpn60.2 gene through recombineering, further suggesting the essentiality of this gene. Hickey et al. (2009) have shown that although both Cpn60.2 and DnaK proteins are present on the bacterial surface, only the former seems to be required to facilitate efficient bacterial interactions with macrophages.

Adhesins as targets for the development of TB control strategies

Significant further research into identifying and characterizing M. tuberculosis adhesins is required to elucidate their precise role in virulence, establish the mechanisms behind their secretion and identify the receptors required for their association with the host cell surface. Uncovering the mechanisms of binding of adhesins to host cells will therefore lead to an improved understanding of the initial events that occur during TB infection. Such studies could potentially result in the development of novel strategies for infection control by means of therapeutics that are able to block secretion and/or prevent reassociation with the cell membrane. Secreted proteins also have potential as biomarkers for diagnostic use, as such molecules are recognized with sensitivity during the humoral response to infection by M. tuberculosis.

Development of anti-adhesives

Greater understanding of the adhesin–ligand interaction may facilitate the design of competitive inhibitors that block adhesin formation, as has been shown in several other organisms, including E. coli. Conjugation of the QFGGN amyloid motif from the E. coli CsgA curlin to proline residues was shown to inhibit bacterial curli formation (Cherny et al., 2005). Bicyclic 2-pyridones, or pilicides, target the chaperone– usher interaction, blocking pilus biogenesis, and leading to an inhibition of adhesion to bladder cells and biofilm formation in E. coli (Pinkner et al., 2006). A multivalent galabiose derivative is an inhibitor of adherence by E. coli P-fimbriae (Salminen et al., 2007). Similarly, α-β-mannose-based inhibitors targeting the FimH adhesin prevent E. coli adhesion on uro-epithelial cells, invasion and biofilm formation (Wellens et al., 2008).

Adhesins as vaccine candidates

The importance of adhesins in pathogenesis implies that disrupting bacterial attachment by specific anti-adhesin antibodies has the potential to incapacitate a pathogen (Klemm & Schembri, 2000). However, the large number of different adhesin genes in an organism’s genome suggests that there are multiple pathways for bacterial adherence (Brzuszkiewicz et al., 2006). This redundancy limits the use of adhesins as vaccine candidates (Kline et al., 2009). In addition, different adhesins are not produced simultaneously and continuously and require different environmental cues for expression, and many exist as antigenic variants (Klemm & Schembri, 2000).

Even with these constraints, adhesins in several species have proven to be useful vaccine candidates, capable of conferring protection in animal models. Their extracellular location is also a favourable feature in that it enables their interaction with protective antibodies (Telford et al., 2006). Their multiple functions in pathogenesis and modulation of the host immune response, together with independent signalling events mediated by individual components of polymeric adhesins, also produces a more robust immune response (Mandlik et al., 2008).

A review by Kline et al. (2009) highlighted adhesive proteins as protective antigens. These include: an E. coli FimH adhesin-based vaccine against cystitis in a primate model (Langermann et al., 2000), the E. coli Dr fimbrial antigen against urinary tract infection in mice (Goluszko et al., 2005), the Salmonella atypical fimbriae B chaperone (SaB) complexed with the SafD adhesin against invasive Salmonella enteritidis infection (Strindelius et al., 2004), a synthetic-peptide consensus-sequence vaccine (C31) against type IV pilus of P. aeruginosa in a mouse model (Kao et al., 2007) and a combination of three group B Streptococcus (GBS) pilus variants that mediate protection in mice against all tested GBS challenge strains (Margaret et al., 2009).

Adhesins as serodiagnostic markers for M. tuberculosis

Exposure to M. tuberculosis antigens leads to the production of specific antibodies, which may be used as markers of infection in serological tests. Several M. tuberculosis antigenic proteins have been evaluated as serological markers, particularly culture filtrate proteins, although surface-exposed proteins appear to be more effective (Abebe et al., 2007).

The 19 kDa lipoprotein adhesin is recognized by sera from TB patients and the sensitivity of this immuno-dominant antigen was found to be 62% in sputum smear-negative TB patients (Jackett et al., 1988; Bothamley et al., 1992a, b; Greenaway et al., 2005). The anchorless adhesin malate synthase is a surrogate marker for TB infection in HIV-seropositive individuals (Abebe et al., 2007), with 57% of TB-infected and 92% of TB-HIV-co-infected patients in
Uganda and South Africa, respectively, showing reactivity to this antigen (Hendrickson et al., 2000). The 30 kDa antigen (antigen 85B or α-antigen) binds to fibronectin and is regarded as a potential M. tuberculosis adhesin (Ratliﬀ et al., 1988). The sensitivity of this antigen, however, was shown to have a broad range of variability, from 41 to 94% (Vikerfors et al., 1993; Lim et al., 1999; Raja et al., 2002, 2004; Uma Devi et al., 2003). Delogu & Brennan (2001) showed that mice infected with M. tuberculosis by aerosolization generated antibodies against the PE_PGRS protein Rv1818. This protein has been suggested to be an adhesin that is involved in both the infection of macrophages and in cell aggregation (Brennan et al., 2001).

HBHA may also be a useful target in TB diagnostics due to its presence in the early stages of TB infection (Masungi et al., 2002). Zanetti et al. (2005) reported that 80% of TB patients (n=5) presented with increasing HBHA antibody titres over a 4-month period. Two of these patients showed a high HBHA antibody level, even though they were tuberculin skin test negative. With regard to the onset of the immune response, methylation of HBHA appears to be crucial for the induction of T-cell antigenicity and protective immunity against M. tuberculosis (Temmerman et al., 2004). Methylated HBHA may thus be a suitable diagnostic marker for identifying individuals with active TB (Abebe et al., 2007).

It was recently shown that the mtp gene, encoding curli-like pili (MTP), is a conserved gene present in M. tuberculosis complex strains, but not in non-tuberculous mycobacteria or other respiratory bacteria (Naidoo et al., 2014). In addition, sera of patients with active TB are known to contain IgG antibodies against MTP (Alteri et al., 2007). MTP may therefore be a suitable marker for the development of a point-of-care TB test.

Conclusions

The involvement of M. tuberculosis surface molecules in adherence is essential to the increased understanding of the bacterium’s pathogenesis. However, little progress has been made in identifying and characterizing the contribution made by the specific adhesins utilized by this pathogen during infection of host cells. This can be attributed to a plethora of factors that include technical diﬃculties in generating gene knock outs, an inability to knock out genes of essential function, a lack of studies on M. tuberculosis entry into non-phagocytic cells, the previous mistaken notion that M. tuberculosis is non-fimbriated and the under-appreciation of the pellicle biofilm lifestyle of this pathogen. Further work on identifying novel adhesins and deﬁning their contribution to TB pathogenesis is critical to increasing our understanding of the physiology and molecular mechanisms of TB pathogenesis that may subsequently facilitate the development of new strategies for TB control.

Acknowledgements

We thank the National Research Foundation (NRF), SA, Medical Research Council (MRC), SA, and College of Health Sciences (CHS), UKZN, for financial support. In addition, Mr S. Ramsugit acknowledged scholarship from the Canon Collins Trust. We also thank Mr Brendan Govender for his assistance in producing the images presented in this publication.

Author contributions

S.R. conceptualized and designed the manuscript; V.S.G. and S.R. drafted the manuscript; M.P. critically revised the manuscript. All authors provided final approval of the article to be published.

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Edited by: S. Spiro