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ENTRY MECHANISMS OF MYCOBACTERIA

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1. ABSTRACT

Since many mycobacteria are facultative intracellular pathogens, their ability to cause disease involves entry, survival and replication within host cells. Despite the fact that mycobacteria were first associated with disease more than 125 years ago, the first step in the production of an infection, entry into host cells, is not well understood. Mycobacteria have the ability to enter a number of different cell types, but the primary cell type that they are thought to replicate within during human disease is macrophages. Since macrophages have a large number of receptors that are designed for relatively non-specific uptake of foreign particles, there are multiple routes by which nearly any bacteria can be taken up. The outcome of mycobacterial entry into macrophages via different mechanisms is unclear. Although it is thought that mycobacteria may enter macrophages by a mechanism that allows them to avoid lysosomal fusion, it remains possible that mycobacteria enter by more than one mechanism, yet remain viable and replicate intracellularly through modification of the phagosome. In the current discussion we will review mycobacterial research specifically relating to the mechanisms of entry into host cells. Although much progress has been made in our understanding of entry by mycobacteria, we anticipate that clarification of the role of entry in pathogenesis will require further application of newly developed molecular tools to dissect each of the proposed mechanisms.

2. INTRODUCTION

Mycobacteria were first associated with human disease in 1874 when Hansen found that a bacterium is associated with leprosy (1). Even today mycobacteria represent some of the most important pathogens of humans and animals. Tuberculosis caused by *Mycobacterium tuberculosis* is currently the number one cause of death worldwide due to a single infectious agent (2, 3). However, *M. tuberculosis* is not the only mycobacterial species that causes respiratory disease. *M. avium* (4, 5), *M. intracellularae* (5, 6), *M. malmoense* (7), *M. bovis* (8), *M. kansasii* (5), *M. xenopi* (5, 9), *M. fortuitum* (5, 9), *M. chelonae* (5, 9), *M. africanum* (10), *M. abscessus* (11), and *M. scrofulaceum* (11) have also been shown to cause respiratory infections. In addition, recent data suggest that *M. leprae* may spread from human to human by aerosol (12, 13). Many pathogenic mycobacteria are facultative intracellular pathogens of monocytic cells; however, they can enter and survive in a number of other cell types. Entry into other cell types may be involved in mycobacterial pathogenesis, for example, *M. avium* has the ability to enter intestinal epithelial cells (14, 16) and *M. tuberculosis* may enter respiratory epithelial cells during early stages of infection (17). Mycobacteria have also been shown to enter microfold (M) cells (18, 19), which raises the question of whether Peyer's patches are one of the portals for dissemination. Since entry is likely to be an important step for growth, dissemination and pathogenesis, the ability to block entry is likely to be an effective mechanism of control for mycobacterial infections. Blocking this step in pathogenesis has the added advantage that it prevents infections early and, most likely, before any clinically relevant pathology has occurred.

Before it is possible to design potential intervention strategies, we must understand the preferred mechanisms used by mycobacteria to enter host cells. Though the entry mechanism used is commonly thought to be important in establishing mycobacterial infections, it is

not well understood. The entry process in other intracellular pathogens is thought to depend upon participation of both the host cell and bacteria (20) and may be controlled by either participant (21). Our understanding of entry into monocytic cells is complicated by their ability to take up both pathogenic and non-pathogenic bacterial species through non-specific uptake mechanisms. In cells of the monocytic lineage, the choice of receptor and mechanism of uptake may determine the subsequent intracellular fate of the bacterium within a potentially hostile environment. In order to better understand this process, we will review the current literature relating to the mechanisms of entry used by different mycobacterial species, with an emphasis on mechanisms observed in cells of the monocytic lineage.

3. COMPLEMENT RECEPTORS AND COMPLEMENT

The first mechanism demonstrated to play a role in the ability of mycobacteria to gain access to the intracellular niche was through opsonization with complement (22) and uptake via complement receptors (23, 24). Complement receptors occur in two distinct structural forms; monomeric transmembrane proteins, such as complement receptor one (CR1) (25), and heterodimeric proteins of the integrin superfamily, including CR3 and CR4 (26). CR3 and CR4 contain identical beta subunits (CD18 or beta2 integrin) and different alpha subunits (CD11b ($alpha_M$) or CD11c ($alpha_x$)). Despite the fact that complement receptors were implicated in mycobacterial entry more than ten years ago, the role of this entry mechanism in mycobacterial pathogenesis remains somewhat controversial. Initial reports demonstrated that the presence of complement enhances the uptake of *M. avium* (22), *M. tuberculosis* (23) and *M. leprae* (24) into human peripheral blood monocytes between two and five-fold. Similar results were obtained using murine peritoneal and alveolar macrophages (27). The involvement of complement receptors was confirmed by inhibiting uptake of complement opsonized mycobacteria with monoclonal antibodies against CR1 and CR3. Inhibition of uptake with these antibodies was around 40% but the combination of two different monoclonal antibodies against CR3 inhibited uptake by more than 80%. These observations led to the conclusions that the CR3 receptor is the predominant receptor used by mycobacteria to enter monocytic cells and that complement opsonization is required for optimal uptake.

Studies with *M. avium* confirmed that complement receptors could be used for entry by this species as well (28). *M. kansasii* has also been shown to enter host cells by a CR3-mediated mechanism that is enhanced by complement opsonization (29). Under opsonizing conditions *M. avium* enters macrophages primarily by CR3, but receptors other than complement receptors appear to be involved (28). In addition, it appears that opsonization with complement is not a prerequisite for mycobacterial entry via complement receptors. Entry through complement receptors can also occur in the absence of serum. *M. avium* can enter human peripheral blood monocytes and alveolar macrophages by a nonopsonic mechanism that involves CR1 and CR3 (30). These observations fit well with the fact that both *M. tuberculosis* (31) and *M. leprae* (24) are thought to have nonopsonic mechanisms for binding complement receptors. Thus, the complement receptor-mediated uptake mechanism appears to be broadly applicable to pathogenic mycobacterial species and appears to occur in both the presence and absence of serum.

Studies designed to demonstrate nonopsonic mechanisms of uptake via complement receptors are limited by the inability to totally remove newly synthesized complement from macrophage tissue culture assays. In order to circumvent this problem other investigators have utilized Chinese hamster ovary (CHO) cells transfected with complement receptors (32, 33). These systems demonstrate the presence of nonopsonic mechanisms of complement receptor binding in *M. tuberculosis*, *M. kansasii* and *M. avium*. However, they were unable to demonstrate enhancement of *M. tuberculosis* binding to CR3 in the presence of complement. This observation suggests that either 1) virulent *M. tuberculosis* strains are primarily opsonized by C3b not C3bi and, hence, can bind CR1 but not CR3 and CR4 or 2) these strains express a lectin-binding site at such high levels that complement-mediated CR3 interactions are negligible and only binding at the CR3 lectin site is observed. The possibility that C3b is the primary opsonin is supported by the observation that mycobacteria interact with C2a to cleave C3 causing an increase in opsonization with C3b (34). Furthermore, in this case the primary receptor for *M. avium* is CR1 rather than CR3. In addition to CR1 and CR3, uptake of *M. tuberculosis* (35) and *M. leprae* (36) can occur via a CR4-mediated mechanism. Transfected CHO cells were also instrumental in demonstrating a role for CR4 in nonopsonic *M. tuberculosis* entry mechanisms (37). Though it has not been determined whether nonopsonic binding to CR4 plays a more important role than opsonic binding to this receptor. There is an overwhelming body of evidence that pathogenic mycobacteria have the ability to bind complement receptors CR1, CR3 and CR4. However, it is unclear whether complement receptor-mediated mechanisms of uptake are critical to the ability of mycobacteria to cause disease.

The role of opsonic mechanisms in initial *M. tuberculosis* infections has been questioned because of the low levels of complement present in the lung (38) despite the endogenous synthesis of complement components by alveolar macrophages and type II epithelial cells (39). Careful examination of the available data suggests that this conclusion may not be warranted. Studies on opsonic mycobacterial entry mechanisms indicate that approximately 1% serum is sufficient for maximal enhancement of mycobacterial entry (23, 24). The level of complement components C4 and C6 in the lungs of healthy nonsmokers is approximately 1% of the levels in their serum (38). These data suggest that although the levels of complement in the lung are lower than in serum, they are sufficient to enhance entry into macrophages by mycobacteria. This conclusion does not; however, mean that complement-mediated mechanisms of entry are critical for pathogenesis.

In order to determine the role of entry by complement receptors in the disease process, investigators have begun to utilize transgenic mouse models of infection (40, 41). One of these studies examined the effects of a CD18 (CR3 and CR4) knockout on the course of *M. avium* infections (41) and the second the effects of a CD11b (CR3) knockout on *M. tuberculosis* infections (40). Neither study found that the mutation had a significant effect upon the course of disease. The *M. tuberculosis* study did, however, demonstrate that at low multiplicities of infection, fewer cells became infected with bacteria (40). This observation may provide some indication of why no significant effects were observed. Both studies utilized high numbers of virulent organisms (>10^5 bacteria) inoculated by tail vein. This contrasts greatly with the presumed course of disease in tuberculosis infections where very few bacteria are thought to be required for infection (42). Thus, additional studies using a more natural route of infection (i.e. aerosol for *M. tuberculosis* and oral for *M. avium*) and lower numbers of infectious organisms are likely to provide important information concerning the role of complement receptors in mycobacterial pathogenesis.

Despite a great deal of research in this area, it remains unclear whether complement receptors are critical for the ability of mycobacteria to cause disease. However, many pathogenic mycobacteria do have the ability to enter via CR1, CR3 and CR4 by both opsonic and nonopsonic mechanisms. There are a number of factors that affect whether complement receptors are used for entry. The growth conditions for (43) and strain of (35, 44) mycobacteria as well as the type of macrophages used (31, 45) play an important role in this determination. It is likely that lavage methods have a significant impact on the physiology of potential host cells. Thus, analysis of the receptors present on alveolar macrophages in fixed lung tissue may be necessary to provide information regarding the state of the cells involved. These studies, though technically difficult, should help to determine whether freshly obtained alveolar macrophages or those maintained in culture for more than four days are representative of the state of these cells *in vivo*. This is particularly important since these alveolar macrophages dramatically differ in their ability to bind mycobacteria (45).

However, the type of macrophages used, may not be nearly as important for determining the mechanism of entry as is the phenotype of the bacteria. In tuberculosis, the primary route of infection is thought to be aerosols produced by coughing. This implies that the bacteria initially infecting alveolar macrophages in the lung are directly obtained from the airways of another individual, where they are presumably produced through replication in alveolar macrophages. Growth in macrophages has been shown to increase mycobacterial adherence (46), invasion (43, 47) and cytotoxicity (46). The mechanism of entry into macrophages used by intracellularly grown mycobacteria is primarily nonopsonic and does not appear to involve complement receptors (43). Based on these observations, examination of mechanisms other than those that are complement receptor-mediated is necessary to better understand the preferred mechanisms of mycobacterial entry into macrophages during natural infections.

4. FIBRONECTIN RECEPTORS AND FIBRONECTIN

Fibronectin receptors have also been implicated in the uptake of mycobacteria into monocytes. Early work demonstrated that mycobacteria had the ability to bind to fibronectin (48, 49), facilitating adherence to the extracellular matrix (50). A potential role in entry into monocytes was suggested when it was found that *M. avium-M. intracellulare* adheres to the $alpha_V beta_3$ fibronectin/vitronectin receptor on monocytes (51). The $alpha_V$ subunit can combine with beta subunits 1, 3, 5, 6 or 8, and most of the resulting combinations can bind to vitronectin, fibronectin and fibrinogen (52). The fibronectin receptor, $alpha_5$ (Itga5), may also be involved, but separation of the activities of this receptor from those of the $alpha_V$ receptors is not straightforward. The fibronectin receptor $alpha_5$ is thought to combine with integrin subunit $beta_7$ (53), but this has not been clearly demonstrated. In mycobacteria it has been suggested that fibronectin-mediated adherence may enhance complement-receptor mediated entry (54). Certainly, the interaction of the *M. avium* GroEL protein with fibronectin receptor(s) enhances complement receptor levels on monocytes (55). Although the interaction of the *M. avium* GroEL with fibronectin receptors is thought to be direct (55), the antigen 85 complex (Ag85A, Ag85B and Ag85C) proteins are thought to interact with these receptors indirectly, via a fibronectin bridge (54, 56). These data suggest that there are at least two different pathways by which mycobacteria can enter host cells using fibronectin receptors.

The involvement of multiple receptors and several potential mycobacterial proteins greatly complicates analysis of the role of fibronectin receptors in entry by mycobacteria. The construction of specific mutants in mycobacteria is necessary in order to dissect the role of each of the potential mechanisms in pathogenesis. Although specific mutants in the Ag85A and Ag85B genes have been constructed by allelic exchange, the effects of these mutations on entry into monocytes have not been examined (57). However, it is likely that single, marked mutations, such as those previously constructed, will not be useful for determination of the role of fibronectin-receptor mediated mechanisms in entry. Multiple mutations are required due the fact that all three Ag85 proteins have similar fibronectin-binding activities (58) and there are actually two Ag85C genes (59). Thus, a single mutant, where only one of these genes is inactivated, may not have a measurable phenotypic effect and the construction of mutations in four genes will require the use of multiple selectable markers with greater risk of having unrelated effects on the bacteria. These effects can be avoided by the construction of multiple unmarked in-frame deletions (60). Since the existing mycobacterial Ag85 mutants have not been evaluated for their effects on entry, it is unclear what role fibronectin-receptor mediated adherence mechanisms play in entry of mycobacteria. The possibility remains that the primary role of these proteins in mycobacterial pathogenesis is in adherence to infected tissues (61) via the

extracellular matrix or biosynthesis of the mycobacterial cell wall (62).

5. SURFACTANT PROTEINS AND THEIR RECEPTORS

Examination of patients with human immunodeficiency virus (HIV), who are thought to be more susceptible to tuberculosis (63, 64), found that surfactant proteins affect entry by mycobacteria into macrophages (65-67). It has been suggested that this enhancement is due to upregulation of other host cell receptors, possibly mannose receptors (68). A link between the expression of receptors for surfactant proteins and mannose receptors has been observed previously (69). However, the fact that one of the surfactant protein receptors, complement component 1q receptor (C1qR), also serves as a receptor for mannose-binding protein (70, 71) and that surfactant proteins themselves bind mannose (72, 73) complicates interpretation of these results. Another surfactant protein A (SP-A) receptor has been identified (74) and is thought to play a role in SP-A-mediated uptake of mycobacteria (74, 75). Thus, in the case of surfactant-mediated uptake of mycobacteria, multiple host cell receptors may also be involved.

It remains unclear whether SP-A-mediated entry mechanisms are advantageous for mycobacteria. One of the primary roles of surfactant proteins in the lung is prevention of potentially damaging immune responses (76-84). However, SP-A is also important in defense against bacterial pathogens by enhancing killing and clearance (85-90). Surfactant protein D appears to play a protective role in mycobacterial infections where it reduces phagocytosis and increases agglutination (91). However, the effects of SP-A on macrophage function remain somewhat unclear, since both stimulatory (87, 92-96) and inhibitory (97, 98) effects have been observed. Similarly, both enhanced killing (99) and survival (100) of mycobacteria have been observed under different conditions after entry via SP-A into macrophages. One explanation for these discrepancies may be the different activation states of the macrophages used. Interferon-gamma primed macrophages were used for the studies demonstrating suppression of reactive nitrogen intermediate production and enhanced mycobacterial survival (100). These data suggest that SP-A may suppress macrophage bactericidal activity later in infections, when the bacteria come in contact with activated macrophages, or in chronically ill patients such as those infected with HIV. At this stage, inhibition of reactive nitrogen intermediate production may serve to protect the host tissues from damage. Overall, based upon current data it appears that the primary role of surfactant proteins, particularly early in infection, is to protect against mycobacterial disease. However, later in the course of infections or in chronically ill patients, SP-A may inadvertently assist in the progression of disease by suppressing the bactericidal activity of macrophages.

6. THE ROLE OF MANNOSE

There are two receptors involved in uptake of mannose-containing particles in macrophages. The macrophage mannose receptor (MR), also known as the insulin-like growth factor II receptor (101-103), mediates attachment to glycoconjugates terminating in mannose, fucose and N-acetylglucoseamine (104-105). MR is thought to be expressed on mature macrophages but only at low levels on monocytes (106). The second receptor is the collectin receptor, also known as the complement component 1q receptor, which interacts with mannose indirectly through mannose-binding protein (71, 107, 108). A role for mannose in entry into macrophages was initially demonstrated for *M. avium*, where mannose-containing compounds were found to inhibit binding to these cells (28, 30). Later studies extended these observations and suggested that there may be a correlation between mannose-mediated uptake and virulence (35).

It has been suggested that the mannose-capped lipoarabinomannan (ManLAM), present in some strains of virulent *M. tuberculosis* (109), plays a role in adherence to macrophages and acts as a ligand for MR (110, 111). The mannosyl units present on ManLAM, and not on AraLAM from non-pathogenic mycobacterial species (112, 113), are thought to be essential for those interactions. These observations contrast with those indicating that both ManLAM and AraLAM can inhibit binding of *M. tuberculosis* to human macrophages (114). These seemingly conflicting observations may be at least partially due to the fact that LAM also has the ability to bind to the CD14 (LPS) receptor (115). In addition, there appears to be cross-talk between the CD14 receptor and the mannose receptor in macrophages (116). It is important to note that no correlation between LAM structure and mycobacterial virulence has been found (117). Furthermore, uptake of LAM by macrophages results in particle delivery to a lysosomal compartment (118). It has been suggested that capsular polysaccharides, other glycolipids such as phosphatidylinositol mannosides (44, 119) or as yet unknown glycoproteins may also be involved in the interaction of mycobacteria with mannose receptors. Despite the presence of multiple potential bacterial ligands, the mannose receptor remains a strong candidate for an important receptor in the uptake of mycobacteria, particularly since uptake via this receptor may reduce oxygen radical production (120). However, this entry mechanism does not appear to be specific to pathogenic mycobacteria (44, 119, 120), suggesting that it may not be critical for pathogenesis.

7. INVASION OF NON-PHAGOCYTIC CELLS

Though considered very successful parasites of macrophages, mycobacteria are also known to enter other cell types. Alveolar epithelial cells outnumber macrophages in the alveolar space and are likely to interact with an aerosolized droplet of *M. tuberculosis*. The ability of mycobacteria to bind and enter epithelial cells has long been documented (121, 122). Despite the possibility that interaction with non-phagocytic cells may play a role in mycobacterial dissemination and pathogenesis, few studies have been conducted in this area. *M. leprae* is an obligate intracellular pathogen that infects Schwann cells, muscle cells, epithelial cells and macrophages. *M. leprae* targets Schwann cells in the peripheral nervous system and the

neurology associated with leprosy is thought to be primarily due to infection of Schwann cells (123). *M. avium* is thought to infect HIV infected individuals through the gastrointestinal tract (124). The gastrointestinal route of infection has been confirmed using animal models for *M. avium* (14, 15). *M. avium* enters epithelial cells efficiently *in vitro* (125-127) and these high levels of entry are not seen with less pathogenic mycobacterial species (126). *M. bovis* BCG has also been shown to traverse the gastrointestinal epithelium of rabbits, where it is thought to use M cells as a portal for entry (18). Furthermore, *M. tuberculosis* can enter M cells in the respiratory mucosa (19) as well as type II alveolar (17) and other types of epithelial cells (126) *in vitro*. Blocking of beta-integrin and vitronectin receptors on alveolar epithelial cells inhibits *M. tuberculosis* adhesion and entry by 80%. *M. tuberculosis* triggers release of TNF-alpha, which may be responsible for increasing the permeability of the epithelial layer (128). Disruption of the epithelium would allow large numbers of bacteria to translocate; thereby, gaining access to the lymphatics and blood stream allowing further dissemination (129). Entry of *M. tuberculosis* into epithelial cells also seems to enhance virulence and bacilli released from these cells may invade macrophages more efficiently (130). These observations suggest that a number of bacterial factors involved in entry are regulated during intracellular growth. The majority of studies on mycobacterial entry have used bacteria grown under standard laboratory conditions, rather than intracellularly. This suggests that there may be an entire spectrum of mycobacterial factors involved in this process that remain to be discovered.

A number of bacterial factors have already been identified that may play a role in entry by mycobacteria into non-phagocytic cells. In the case of *M. leprae*, phenolic glycolipid is thought to be involved via its ability to interact with laminin (131, 132) and alpha-dystroglycan in Schwann cells (133). Phosphatidylinositol mannoside can mediate adhesion of *M. tuberculosis* to fibroblasts and endothelial cells (119). In addition, several mycobacterial proteins have been implicated in adhesion (127, 134) and entry (126) into epithelial cells for *M. avium*. However, none of these determinants have been well characterized and no mutations have been constructed in the bacterial genes involved. The best-characterized gene that may play a role in entry into non-phagocytic cells is designated *mce* for mammalian cell entry (135). Unfortunately, the original genomic fragment (GI #44606) that confers the entry phenotype is non-contiguous, consisting of at least five different fragments of the *M. tuberculosis* genome (136). However, the activity of this fragment has been attributed to a 636 bp region internal to the *mce1* gene, the first of four *mce* genes that are present in *M. tuberculosis*. Each of the *mce* genes is present within a putative eight-gene operon where the *mce* gene is third (136). Similar genes have been found in *M. leprae* (137), *M. avium*, *M. intracellulare* and *M. scrofulaceum* (138). Interestingly, most of the *mce3* operon is missing from *M. bovis* BCG; whereas, the other three copies of this operon are intact (139). A mutant constructed in the *mce1* gene of *M. bovis* BCG, displays a defect in entry into epithelial cells after 4-8 hours of infection (140). While these results are certainly intriguing, it is unclear that the *mce1* gene is responsible for the phenotype since an insertion mutation was used, which would have polar effects on the five genes downstream. In addition, when assayed at such late time points after addition of the bacteria, it is difficult to ascertain whether the *mce1* gene plays a role in entry or survival in these cells. Lastly, since this mutation has not been complemented, it remains possible that a secondary mutation elsewhere in the chromosome, arising during genetic manipulation, is responsible for the phenotype. Clearly, further studies are necessary to demonstrate a crucial role for these genes in entry. Further examination of the mechanisms involved in entry into non-phagocytic cells by mycobacteria is likely to provide important insight into how mycobacteria disseminate to other tissues from the primary site of infection.

8. PERSPECTIVE

There are a number of potential mechanisms that mycobacteria can use to enter host cells. The presence of a large number of pathways is particularly evident in cells of the monocytic lineage (figure 1). Interestingly, the majority of the receptors that have been investigated, thus far, are commonly used by macrophages to kill potentially dangerous bacteria in a non-specific fashion. Clearly, the innate immune response of humans has developed elegant systems for the removal of invading bacteria without a need for specific recognition. This observation suggests that bacterial entry into macrophages via these receptors primarily results in killing and degradation. Somehow mycobacteria must have the ability to either avoid or resist the bactericidal activity that would normally occur. There are a number of potential pathways by which mycobacteria might circumvent the bactericidal mechanisms of macrophages: 1) mycobacteria interact with a number of different receptors for adhesion, but an as yet unidentified receptor leads to an advantageous uptake mechanism; 2) mycobacteria interact with one or more of the previously identified receptors in an unusual manner that affects signaling leading to an advantageous uptake mechanism; 3) mycobacteria secrete factors prior to entry that affect signaling pathways in the host cell leading to an advantageous uptake mechanism via any receptor; 4) mycobacteria can enter host cells through a number of non-specific mechanisms followed by modification of the intracellular compartment to allow survival. Survey of the current literature suggests that a number of different entry mechanisms can lead to intracellular survival. However, it will be necessary to construct specific mutations in the genes involved in each of these potential entry mechanisms and assess their phenotypes in both resistant and susceptible animal models to properly evaluate these alternative hypotheses.

In addition to a role for host factors in the importance of different entry mechanisms, growth conditions for the bacteria are also critical. In natural infections one would assume, at least in the case of *M. tuberculosis*, that the bacteria are released from an

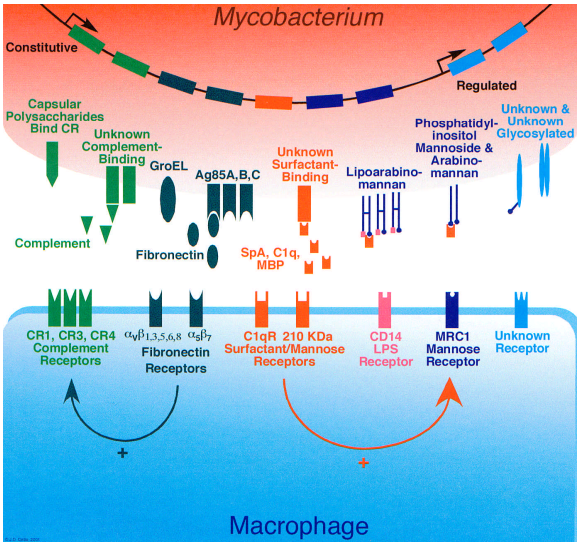

Figure 1. Potential mechanisms used by mycobacteria to enter macrophages. Each of the components shown represents either a bacterial ligand, opsonin or a potential host cell receptor. A complex combination of these factors is likely to play an important role in the ability of mycobacteria to trigger a preferred mechanism of entry. Arrows and plus symbols in the macrophage represent pathways where activation or binding to one receptor has been shown to affect the activity or expression of a second receptor or class of receptors. Arrows in mycobacteria represent regulons of genes involved in entry that are either expressed under standard laboratory growth conditions (constitutive) or regulated by intracellular growth (regulated). Many of the interactions proposed in this model have not been characterized fully, but are put forward as possibilities based on the available data. Abbreviations: CR, complement receptor; SpA, surfactant protein A; C1q, complement component 1q; C1qR, C1q receptor; 210 KDa, the uncharacterized 210 KDa protein demonstrated to bind surfactant protein A (74); MBP, mannose-binding protein; Ag85A, B, C, antigen 85A, 85B and 85C; LPS, lipopolysaccharide; MRC1, macrophage mannose receptor.

intracellular growth environment prior to infecting a new host. Intracellular growth of mycobacteria appears to significantly affect the mechanisms of entry used (43, 46, 47). These observations suggest that a large number of the mycobacterial factors involved are yet to be determined. In addition, these as yet unknown factors are likely to be the most relevant to the mechanisms used by mycobacteria in natural infections. By further examination of the genes involved in entry of intracellularly-grown mycobacteria it may be possible to eliminate at least some of the potential entry mechanisms previously identified and narrow the scope of future research. A number of the previously identified entry mechanisms have been blocked for *M. avium* (30) and *M. tuberculosis* (141) without preventing the ability of the bacteria to survive intracellularly. Both of these studies support the concept that there are a number of different mycobacterial factors that may be involved in entry and have not yet been identified. Clearly, there is a great deal of work that remains to be done in order to understand the mechanisms involved in entry into host cells by mycobacteria. With the rapid advancement of molecular tools to understand both the host and pathogen involved, we expect that great strides will be made in this area of research in the near future.

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