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Review

Mechanisms of *Mycobacterium avium* Pathogenesis

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Abstract. Infections caused by *Mycobacterium avium* are common in AIDS patients and patients with chronic lung diseases. The bacterium can be acquired both through the intestinal route and respiratory route. *M. avium* is capable of invading mucosal epithelial cells and translocating across the mucosa. The bacterium can infect macrophages, interfering with several functions of the host cell. The host defense against *M. avium* is primarily dependent on $CD4^+$ T lymphocytes and natural killer cells. Activated macrophages can inhibit or kill intracellular bacteria by mechanisms that are currently unknown, but *M. avium* can invade resting macrophages and suppress key aspects of their function by triggering the release of transforming growth factor β and interleukin 10. Co-infection with HIV-1 appears to be mutually beneficial, with both organisms growing faster.

Key words: *M. avium*; pathogenesis.

Introduction

Mycobacterium avium is an environmental microorganism that can be found in water reservoirs as well as natural sources of water and soil[45, 51]. *M. avium* is also encountered infecting birds and pigs. The group of the *M. avium* complex includes *M. avium, M. intracellulare* and *M. scrofulaceum*. While *M. avium* has been identified as a common pathogen in patients with acquired immunodeficiency syndrome (AIDS), both *M. avium* and *M. intracellulare* have been isolated from the lungs of patients with chronic lung diseases[42, 78]. More recently, cases of *M. avium* lung infection have been identified in women with chest deformities[52] and in patients with cystic fibrosis[3]. In addition, familiar cases of *M. avium* infection have been described in patients with an absence of interferon γ (IFN-γ) receptor[40] as well as interleukin 12 (IL-12)[4].

M. avium infection occurs by at least two routes: 1) following ingestion, the bacteria survive the acidic pH of the stomach and subsequently colonize the intestinal tract. Colonization is followed by translocation across the intestinal mucosa; 2) aerosol transmission similar to tuberculosis.

Interaction between *M. avium* and the Intestinal Mucosa

Among the organisms of the *M. avium* complex (*M. avium, M. intracellulare* and *M. scrofulaceum*), *M. intracellulare* is the pathogen most commonly isolated, causing lung infection (but not exclusively) in patients with chronic lung diseases, such as bronchiectasis. Neither *M. intracellulare* nor *M. avium* have been shown to invade lung epithelial alveolar cells with great efficiency *in vitro*[13], which suggests that lesions of the bronchial epithelium or dilation of the alveolar sac with

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consequent fibrosis may be required in order for the bacteria to cause disease. In addition, to this date we do not understand why *M. intracellulare* predominates among the organisms of the *M. avium* complex as a lung pathogen. In contrast, AIDS is associated with disseminated *M. avium* infection and, in this case, it seems that the majority of the infections are acquired through the gastrointestinal route (although some *M. avium* bacteremia are linked to the respiratory route)[36, 53]. Previous epidemiologic observations indicate that the presence of a functional stomach represents a mechanism of host defense against *M. tuberculosis*[35]. However, our findings showed that, in contrast to *M. tuberculosis* and the commensal bacterium *M. smegmatis*, *M. avium* strains resist the effects of the acidic environment of the stomach (pH 2.2)[32]. The data also suggest that incubation in a hypoosmolar condition (natural *M. avium* environment) increases the ability of the bacterium to survive in an extremely acidic environment[32].

A number of studies have shown, both *in vitro* as well as *in vivo*, that *M. avium* can enter the intestinal epithelial mucosal cells with significant efficiency[29, 59]. When given to mice orally, *M. avium* enters the intestinal mucosa preferentially at the terminal ileum of the intestine and, subsequently, causes disseminated disease[20]. The observation that the great majority of *M. avium* invades the intact mucosa of the intestinal tract in the terminal ileum suggests that *M. avium* uses the Peyer's patches to translocate the mucosa in a manner similar to *Salmonella*, *Shigella*, *Listeria*[54] and other intestinal pathogens. In fact, previous observations have demonstrated that both *M. bovis* BCG[44] and *M. paratuberculosis*[60] move across the intestinal mucosa by using specialized cells present in the Peyer's patches, the M cells. However, our observations suggest that *M. avium* crosses the intestinal mucosa primarily by entering enterocytes, although a few bacteria are seen within M cells[22]. The environment within the intestinal lumen is also capable of influencing gene expression in *M. avium* and recent studies have shown that both hyperosmolarity and low tension of oxygen are associated with a significant increase in the ability of *M. avium* to enter epithelial cells *in vitro*[19].

Studies have proposed that *M. avium* enters the intestinal mucosal cells by using the mucosal cell's fibronectin to bind to the integrin receptor[72]. A fibronectin attachment protein (FAP) has been cloned in *M. avium* and antibodies against it prevent *M. avium* association with bladder epithelial cells[72]. Interestingly, other mycobacteria, such as *M. tuberculosis*, *M. leprae* and BCG[72], share the same fibronectin attachment protein, although only BCG has been associated with the intestinal route of infection. Other studies have identified a 23 kDa protein of *M. avium* that increases the association of the bacterium with epithelial cells; however, no role has been established regarding the association between *M. avium* and the intestinal mucosal cell[23].

Following invasion of the intestinal epithelial cells, *M. avium* is found within cytoplasmic vacuoles that do not acidify[64]. The vacuoles, when large in size, contain several microorganisms, but they eventually segment and 2 or 3 days after uptake each bacterium is usually present alone in a vacuole[64]. The *M. avium*-containing vacuoles migrate to the peri-nuclear region and, 24 to 48 h, following infection, there is a concentration of intracellular organelles in areas surrounding the mycobacterial vacuoles[64]. Opaque material can be detected within the mycobacterial vacuole, but the meaning is presently unknown.

M. avium entry into epithelial mucosal cells is neither accompanied by chemokine production nor associated with cell infiltration during the first days after infection[57], the opposite of what has been shown with other microorganisms, such as *Salmonella*[55, 77]. In fact, after oral administration of *M. avium* to mice, approximately one week is needed before cell infiltration can be detected in the invasion sites[57], suggesting that no significant release of chemokines occurs. Experiments *in vitro* have confirmed the hypothesis that *M. avium* infection of epithelial cells suppresses the production of IL-8 and RANTES, even during co-infection with *Salmonella*[65]. Therefore, our current knowledge suggests that *M. avium* invades the intestinal mucosa but in a quiet manner which allows it to establish an infectious niche before it has to face the host's immune response.

Interaction with Macrophages

Once in the host, *M. avium*, similar to other pathogenic mycobacteria lives within tissue macrophages. It seems that *M. avium* is adapted to the environment of macrophages and, therefore, the ability of these bacteria to enter macrophages is crucial for survival.

Several mechanisms have been described as being responsible for the entry of mycobacteria into phagocytic cells. The use of the complement receptor CR3 (CD11b/CD18) has been reported to be important for the phagocytosis of *M. avium* and *M. tuberculosis*[30, 70]. Mycobacteria bind to the CD11b portion of the molecule in the absence of the C3b and C4b fractions of the complement[70]. In addition, other receptors in human

macrophages, such as CR1, and mannose receptors have been shown to have a participation in the process of internalization[30, 69]. More recently, it was proposed that a novel mechanism was responsible for the uptake of *M. avium* by macrophages. As part of this mechanism, *M. avium* would cleave a C2a molecule and utilize it to bind macrophages[71]. This mechanism would be more important in tissues where serum can be present, but its role is less certain in, for example, the lung mucosa, where complement factors are not readily available.

Studies using CD18 knock-out mice have suggested that *in vivo* (in contrast to the *in vitro* findings) CR3 and CR4 receptors do not participate in the uptake of *M. avium* by macrophages[14]. Infection in mice lacking the CR3/CR4 receptors resulted in similar numbers of bacteria in organs and electron microscopy confirmed the intracellular presence of these bacteria. This study confirmed a study *in vitro* which demonstrated that intracellular (macrophage) *M. avium* leaves the host cells following apoptosis and subsequently invades an uninfected monocyte or macrophage by using a complement-independent receptor[17]. Infection of the "second" macrophage is dependent on the binding of both scavenger receptors and transferin receptors[17]. This phenomenon is very likely to occur *in vivo*, where bacteria multiply intracellularly and subsequently exit the "first" macrophage to invade a "second" macrophage. This sequence of events explains the dissemination of intracellular pathogens.

Once inside a macrophage, *M. avium* has a unique manner of interfering with the cell's trafficking. *M. avium* lives in cytoplasmic vacuoles that characteristically do not acidify and do not fuse with lysosomes[43, 74]. *M. avium* vacuoles are immature and contain markers that suggest an intermediary stage between early and late endosome[43, 74]. These vacuole characteristics are also shared by vacuoles of cells infected with *M. tuberculosis*[34, 37] and *M. marinum*[6].

Other mechanisms that allow for the *M. avium* survival within macrophages are not well known. A recent observation showed that, once in a macrophage, *M. avium* shifts the metabolism with up-regulation of the enzyme isocitrate lysase[9] that is part of the glycoxylate bypass. The expression of isocitrate lysase may be a consequence of a possible intracellular anaerobic environment. Our laboratory has recently identified a number of *M. avium* genes that are preferentially expressed intracellularly, such as a transcriptor regulator *trgR*[63]; however, the exact role of the *trgR* gene in pathogenesis is currently unknown. In addition, we cloned *fecB*, a gene encoding for a lipoprotein involved in the transport of iron by the bacterium[76]. A possible clue to the participation of *fecB* in virulence mechanisms is its absence in the non-pathogenic mycobacterium, *M. smegmatis*[76].

The importance of the concentration of intravacuolar iron has been emphasized by the genetic linkage of polymorphisms in and around the natural resistance-associated macrophage protein 1 (Nramp1) locus with the susceptibility to tuberculosis[8] and leprosy[1] in humans. Nramp1 is a member of a gene family that encodes transport proteins for divalent cations, and Nramp2 has been implicated to be involved in the iron transport in intestine and other tissues[48]. In macrophages, Nramp2 is primarily detected in recycling endosomes and also, to a lesser extent, at the plasma membrane, colocalizing with transferring, suggesting a role for transporting Fe^{2+} into the cytoplams after acidification of the transferrin-positive endosome[48]. Nramp1, in contrast to Nramp2, is only expressed in the macrophage and is localized in the late endosomal and lysosomal compartment where it may function: 1) as a pump that depletes the phagosomal compartment of these nutrients, especially of iron[7]; 2) as a fusogen that promotes vesicle fusion resulting in recruitment of vacuolar H^+-ATPase activity[49]; and 3) as a mediator of microtuble-dependent phagosome and/or lysosome transport[75]. Interestingly, a homolog to the *Nramp* gene has been identified in the *M. tuberculosis* genome that may compete in the phagosome for divalent cations[2].

Mechanisms of Host Defense against *M. avium*

All the evidence indicates that "healthy" individuals are able to control *M. avium* infection, and only when a localized or systemic impairment in the immune defense develops is *M. avium* able to cause disease. As an environmental organism, *M. avium* is ingested with water and food, but also is inhaled with aerosols. Therefore, a large majority of the population has contact with the bacterium, but never develops disease. What then makes patients with AIDS, chronic pulmonary conditions, hairy cell leukemia, elderly women and many individuals without a detectable predisposing cause more susceptible to the disease? Although we now know some possible answers, we are still far from having definitive answers.

Years ago, Orme and Collins demonstrated the importance of specific CD4 T cells in the host response against *M. avium*[50]. In fact, their findings agree with the observation that AIDS is a condition that facilitates *M. avium* disease. By the same token, studies over the

years have confirmed the role of natural killer (NK) cells in the non-specific response against *M. avium*[15, 28], both in mice as well as in human systems[15, 28]. NK cells respond to macrophage cytokines such as IL-12 and tumor necrosis factor-α (TNF-α) with the production of IFN-γ, TNF-α and granulocyte macrophage colony-stimulating factor (GM-CSF). Then, infected macrophages, when stimulated by NK cell products, have been shown to control the intracellular infection[31]. Cytotoxicity is not a mechanism by which NK cells participate in the host defense against *M. avium*, although direct contact with macrophages increases the ability of NK cells to stimulate macrophages[15, 31].

In contrast to CD4 T cells, the role of $CD8^+$ T cells has not been demonstrated. Studies using cytotoxic antibodies as well as the use of mice that lack the presence of $CD8^+$ T cells have indicated that $CD8^+$ T cells do not have an essential role in the host defense against *M. avium*[18, 39, 67]. Whether $CD8^+$ T cells are important at some level in the absence of $CD4^+$ T cells is currently unknown.

Macrophages are certainly the central piece in the defense against *M. avium*. Although *M. avium* (as well as *M. tuberculosis*) posseses mechanisms to survive in macrophages, activated phagocytic cells can efficiently eliminate the bacterium. Several aspects of the interaction between *M. avium* and macrophages are now known. Macrophages stimulated with cytokines such as TNF-α, IFN-γ or GM-CSF[25, 27] can control the infection. These observations made initially *in vitro*, were subsequently confirmed in experimental mice[5, 16, 24], and GM-CSF has been used in patients with untreatable *M. avium* disease with success[56]. We also know that IL-12 is a key cytokine in the host defense against mycobacteria and that lack of IL-12 is associated with severe infection in both mice[33, 68] and humans[58]. In fact, therapy of mice with disseminated infection using IL-12 resulted in significant improvement[21]. However, the line between efficacy and toxicity of IL-12 is a fine one, and association of a macrolide (clarithromycin) which has interesting anti-inflammatory properties eliminated the IL-12-related toxicity without affecting the beneficial effect[21].

The pathways and mechanisms used by activated macrophages to kill *M. avium* are not well known. Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) production are toxic to some but not to the majority of *M. avium* strain[26, 66]. In addition, O_2^- and H_2O_2 are only produced in large amounts when the bacterium is internalized using the Fc receptor, but not when the uptake occurs through other receptors[79]. *M. avium* has a superoxide dismutase (sodA) and a catalase which has the expression controlled by oxyR[41]. These enzymes may have a role in suppressing O_2^- and H_2O_2 production. The production of nitrates such as nitric oxide was suggested initially as a mechanism of *M. avium* killing[38], but subsequent studies have shown that nitrogen intermediates are not involved in the killing of virulent *M. avium*[10]. More recently, work using nitric oxide knock-out mice has confirmed the observation *in vitro* that this pathway is not involved in *M. avium* killing[46]. Other bactericidal products in phagocytic cells are small peptides called defensins. Incubation of *M. avium* with purified defensins from rabbit neutrophils *in vitro* resulted in significant killing of mycobacteria[62]; however, the fact that macrophages do not produce significant amounts of defensins and that mycobacterial phagosomes do not fuse with lysosomes (when defensins are present) make the importance of this mechanism unlikely.

Mycobacterial phagosomes in activated macrophages, different from phagosomes in resting macrophages, fuse with lysosomes and, although an acidic environment does not kill *M. avium* during short exposure[32, 46], it is plausible to expect that long exposure to acid associated with the action of lysosome enzymes may have a lethal effect on the bacterium.

Nonetheless, the efficacy of activated macrophages is not observed in resting cells. It seems that once *M. avium* invades a resting macrophage it paralyzes vital functions of the cell, securing itself a proper environment for survival. Among the responses triggered by *M. avium* when entering macrophages is the production of suppressor cytokines, such as transforming growth factor β (TGF-β) and IL-10[11, 12, 47]. A number of groups have now shown that this is initiated right after uptake and that this strategy is very effective in suppressing macrophage function.

The release of TGF-β, for example, is induced by virulent but not by attenuated *M. avium* and the TGF-β released from macrophages following *M. avium* infection is from the cytoplasmic storage[11], therefore not requiring protein synthesis. This strategy ensures that macrophages infected by *M. avium* will secrete large quantities of TGF-β shortly after infection. In addition, TGF-β is usually secreted from macrophages in the inactive form, but *M. avium* infection induces the release of active TGF-β, suggesting that either *M. avium* itself or an enzyme released by infected macrophages cleaves TGF-β to the active form[11]. The consequence is that resting macrophages infected with *M. avium* become cells incapable of responding to the presence of the bacterium and incapable of participating in efforts to eliminate the infecting organism.

It is now clear that co-infection of macrophages with HIV-1 virus and *M. avium* results in increased growth of both the virus and the bacterium[61, 73].

In summary, *M. avium* is a very specialized bacterium that can invade and survive in both macrophages and epithelial intestinal cells through mechanisms that impair the ability of the host to detect and fight the infection.

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