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MINIREVIEW

Tuberculosis: Latency and Reactivation

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Tuberculosis is a major cause of death around the world, with most of the 1.5 million deaths per year attributable to the disease occurring in developing countries. This disease is caused by *Mycobacterium tuberculosis*, an acid-fast bacillus that is transmitted primarily via the respiratory route. Infection occurs in the lungs, but the organism can seed any organ via hematogenous spread. There are various possible outcomes for a person encountering *M. tuberculosis* bacilli. First, the bacillus can be immediately destroyed by the host's innate responses. However, the innate mechanisms that protect against infection are largely uncharacterized; obviously, this is a very important area of study for vaccine development. Second, a proportion of persons infected with *M. tuberculosis* develops active tuberculosis within a finite time frame (1 to 3 years) (74). This group presumably lacks the ability to both control the initial infection and develop a protective response in time to prevent disease. Finally, it is generally thought that the majority of persons infected with *M. tuberculosis* have a clinically latent infection; that is, they are infected and purified protein derivative(PPD)-positive by skin test but do not present with clinical symptoms and are not contagious to others. However, a number of studies indicate that some infected persons revert to a PPD-negative status, giving weight to the argument that elimination of the organism by the host has occurred (33, 73). In approximately 5 to 10% of latently infected persons, the infection will reactivate and cause active tuberculosis (71). It has been estimated that up to one-third of the world's population is infected with *M. tuberculosis*, and this population is an important reservoir for disease reactivation (21). Understanding latent and reactivation tuberculosis, at the level of both the host and the bacillus, is crucial to worldwide control of this disease.

Infection with *M. tuberculosis* is believed to occur in an alveolar macrophage initially. The bacteria replicate within the macrophage and induce cytokines that initiate the inflammatory response in the lungs. Macrophages and lymphocytes migrate to the site of infection and form a granuloma (18). The function of the granuloma is to segregate the infection to prevent spread to the remainder of the lung and to other organs, as well as to concentrate the immune response directly at the site of infection. The granuloma is maintained in a persistently infected host, probably due to chronic stimulation of the immune cells, and forms the basis for a tuberculous lesion. Live bacilli have been isolated from granulomas or tubercles in the lungs of persons with clinically inactive tuberculosis, indicating that the organism can persist in a granulomatous lesion for many years (57, 67).

At the most fundamental level, latent tuberculosis can be viewed as an equilibrium between host and bacillus. In response to infection with *M. tuberculosis*, most persons mount a robust immune response, culminating in the formation of a granulomatous lesion that apparently contains the infection. The host response prevents active disease from occurring, and the bacterium avoids elimination. In most cases, the host response is sufficient to forestall active disease for a lifetime. However, occasionally the immune response fails in some way and the infection reactivates to cause active disease. There are a number of important questions that remain to be answered with respect to latent tuberculosis. How does the host control the initial infection to prevent disease? What immune factors contribute to establishment of a latent infection? Which immunologic components are required to maintain a latent infection and prevent reactivation? How does the bacterium evade host antimcrobial defenses and survive in the face of a strong immune response? Is the bacillus dormant, slowly or intermittently replicating, or metabolically active? All of these questions impact another question relevant to vaccine development: How can the immune system be induced to eliminate, rather than just control, the tubercle bacillus? Otherwise, immune compromise can lead to reactivation of the infection. A clearer picture of the interactions between host and bacillus during latent or persistent infection is essential to answering these questions. The present status of research in these areas is the subject of this review.

ANIMAL MODELS

Studies of latent tuberculosis have been hampered by the lack of animal models truly representative of human latent tuberculosis. Of course, without a concrete picture of human latent *M. tuberculosis* infection, it is difficult to model it in an animal system. The most commonly used animal model for studying tuberculosis is the mouse. When an appropriate dose is used, *M. tuberculosis* delivered via the aerosol or intravenous route grows relatively unimpeded in the lungs (and spleen) of mice for the first 2 to 4 weeks (reviewed in reference 59), at which point in relatively resistant mouse strains (such as C57BL/6) the immune system controls the growth of the bacteria and bacterial numbers reach a plateau ($\sim 10^5$ to 10^6 CFU/

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lung). Of interest is the failure of the immune response to substantially reduce the bacterial numbers in the lung or spleen after this point; in fact, a persistent or chronic infection ensues and is maintained for many months (27, 58). Despite relatively high levels of bacteria in the lungs of the mice and pathology associated with the infection, the mice do not show clinical signs of disease and can survive for >1 year postinfection. This persistent-infection state has been used as a model of latent tuberculosis by a number of groups (1, 27, 54, 58, 70). It is an attractive model in that it truly represents equilibrium between host and bacillus and in that the bacterial numbers are controlled by the immune response, which is true for the human latent infection. Induction of immune compromise results in reactivation or relapse of the persistent infection. However, the high bacterial numbers in the lungs of the mice probably are not reflective of the situation in humans. There are data suggesting that the bacteria may be in a quiescent state (i.e., not actively replicating) during the persistent phase (66). This model has been effective in studying persistent tuberculosis, with some of the findings from this model applicable to human latent and reactivation tuberculosis, as described below.

Another mouse latent tuberculosis model was developed by McCune and colleagues at Cornell University in the 1950s (48–50). The Cornell model involved treating *M. tuberculosis*-infected mice with antimycobacterial drugs, which reduced the bacterial burden to undetectable levels. After antibiotic treatment, reactivation of the infection can occur spontaneously or in response to immunosuppressive agents, such as glucocorticoids. This model is attractive because of the low bacterial burden in the mice. However, introduction of antibiotics to effect this reduction in bacterial numbers does not mimic the situation in natural human latent tuberculosis and may affect the development of a protective immune response. This is particularly true for the early studies with this model, where antibiotics were administered immediately after infection. In addition, the effects of long-term antibiotics on the growth or survival characteristics of organisms in vivo introduces an additional variable (69). A recent publication describes the testing of various modifications of the Cornell model, with the conclusion being that this model was technically difficult, expensive, and unpredictable for studies on the immunologic basis of latent and reactivation tuberculosis (69). However, others have used variations on this model to study persistence and reactivation of mycobacteria in the host, as well as vaccination of latently infected hosts (7, 40, 42, 52, 61).

Other animal models for the study of tuberculosis include guinea pigs, rabbits, and nonhuman primates, although latency models developed with these experimental animals have not been widely used at this point. Despite the difficulty in modeling human latency in experimental animals, the understanding of both host and microbial factors that contribute to the establishment and maintenance of a persistent *M. tuberculosis* infection has progressed and the information gathered is pertinent to human latent tuberculosis.

HOST RESPONSES IMPORTANT IN LATENT AND REACTIVATION TUBERCULOSIS

Murine models have been used extensively to delineate the host factors that are key to controlling the initial infection with *M. tuberculosis*. Using either gene-deficient (knockout) mice or neutralization with antibodies, various cytokines and cell types have been demonstrated to be essential to the control of *M. tuberculosis*. T cells, both $CD4^+$ and $CD8^+$, participate in protection against tuberculosis (reviewed in reference 24). These cells function to activate and destroy *M. tuberculosis*-infected macrophages. Production of reactive nitrogen intermediates (RNI) by induction of nitric oxide synthase (NOS2) in macrophages is necessary to protect mice against tuberculosis (11, 43); there is now a fair amount of evidence to indicate a role for RNI in human tuberculosis as well (reviewed in reference 10). A pivotal cytokine in the immune response to this pathogen is gamma interferon (IFN-γ). Mice deficient in the gene for IFN-γ are the most susceptible to fatal tuberculosis reported to date (15, 23). This cytokine is responsible for macrophage activation in tuberculosis (17, 23), including the production of RNI, which is the only known mechanism by which macrophages can kill intracellular *M. tuberculosis* (12). However, it is likely that there are additional mechanisms by which IFN-γ contributes to control of tuberculosis, since IFN-$\gamma^{-/-}$ mice are more susceptible than mice deficient in NOS2 (11, 23, 43). Humans deficient in the gene for IFN-γ or the IFN-γ receptor also show enhanced susceptibility to infections with mycobacteria, including *M. tuberculosis* (reviewed in reference 60).

Tunor necrosis factor alpha (TNF-α) is also a crucial cytokine for control of acute tuberculosis in mice. Without this cytokine, effective granuloma formation is diminished and bacterial numbers rapidly increase, resulting in death of the mice (2, 25). The effects of TNF-α on the response to *M. tuberculosis* are multifaceted and include macrophage activation and RNI production, granuloma formation, and possibly induction of pathology (2, 25, 41). A recent study, in which high levels of TNF-α from recombinant *Mycobacterium bovis* BCG caused excessive pathology, supports the hypothesis that the amount of TNF-α in the lungs during infection determines whether the cytokine is protective or destructive (3). However, recent data obtained with a persistent infection model indicate that a relative deficiency of TNF-α can also result in destructive immunopathology (54). In that study, TNF-α was neutralized in mice that had previously been infected with *M. tuberculosis* for 6 months. The loss of TNF-α resulted in a modest increase in bacterial numbers, but destructive and aberrant pathology discordant with the number of bacteria in the lungs. Loss of granulomatous structure, as well as intense inflammation culminating in keratin deposition and squamous metaplasia in the lungs was observed. The unfocused T-cell and macrophage infiltrate in the lungs of the anti-TNF-α antibody-treated mice may contribute to the exacerbation of pathology. Interestingly, significant necrosis was not observed in those mice; necrosis appears to be correlated with high bacterial numbers in murine tuberculosis and was present in acute infections in TNF-α-deficient mice (25). In a modified Cornell model, neutralization of TNF-α also led to reactivation of infection in a subset of mice (69). Although bacterial numbers did not reach high levels in these TNF-neutralized and reactivated-infection Cornell model mice, similar aberrant pathology was observed (54). In a separate study, mice with a persistent *M. tuberculosis* infection were coinfected with adenovirus producing soluble TNF receptor, effectively neutralizing endogenous TNF in the

lungs; bacterial loads increased dramatically in these mice, and they succumbed to fatal tuberculosis (1). Together, these studies revealed a major role for TNF-α in the control of persistent infection and modulation of the pathologic response to *M. tuberculosis*. The mechanism by which this cytokine organizes the localized cellular response in a chronic infection remains to be determined, but the effects of TNF-α on adhesion molecule, chemokine, and chemokine receptor expression are likely to contribute to this function. TNF-α can also affect cytokine expression, which may impact granuloma formation and function. Anti-TNF-α antibodies are currently in clinical trails for treating various conditions, including rheumatoid arthritis. Neutralization of this cytokine should be approached with caution, since the effects on the immune response to infections can be profound. In fact, in at least one clinical trial, a subject receiving anti-TNF-α antibody administration experienced disseminated *M. tuberculosis* infection (45).

Both IFN-γ and TNF-α contribute to resistance to *M. tuberculosis* in part by their roles in activation of macrophages and induction of NOS2 expression (reviewed in reference 22). In a persistent *M. tuberculosis* infection, these cytokines continue to be produced in the lungs of mice, suggesting that continuous macrophage activation is important in preventing reactivation of the infection (27). NOS2 mRNA and protein is also present throughout the persistent infection (27, 70). Inhibition of NOS2 activity [with aminoguanidine or L-N^6-(1-iminoethyl) lysine (L-NIL)] in persistently infected mice led to rapid increases in bacterial numbers in the lungs, although there was little effect observed on bacterial numbers in the liver and spleens of these mice (27). This was in contrast to acute infections in NOS2$^{-/-}$ mice or in mice treated with NOS2 inhibitors, where increases in bacterial numbers were observed in all three organs (11, 44). Inhibition of NOS2 activity in long-term-infected mice treated with antibiotics (a modified Cornell model) also led to reactivation of the infection (27). These data indicate that continuous macrophage activation and RNI production is important in preventing reactivation in the lungs. The contribution of IFN-γ to the control of a persistent infection has been difficult to test, since the anti-IFN-γ antibodies tested have not been entirely successful, even in an acute infection (J. L. Flynn, unpublished data). However, treatment of Cornell model mice with anti-IFN-γ antibodies did result in reactivation of the latent infection (69, 76), although there was spontaneous reactivation, albeit at a slower rate, in the control mice (69).

T cells play an important role in the immune response against *M. tuberculosis*. Both CD4 and CD8 T cells participate in control of acute tuberculosis in mice (reviewed in reference 24), although the relative importance of CD8 T cells is more controversial (53). In humans, infection with human immunodeficiency virus (HIV) leads to a loss of CD4 T cells, which is associated with an increased risk of tuberculosis. While an HIV-negative, PPD-positive person has a 10% lifetime risk of developing active tuberculosis, coinfection with *M. tuberculosis* and HIV carries a 5 to 15% yearly risk of active tuberculosis (71). It is generally accepted that a TH1 response, characterized by production of IFN-γ by CD4 T cells, is important in control of *M. tuberculosis* infection. The majority of reports on *Mycobacteria*-specific CD4 T cells isolated from PPD-positive or active tuberculosis patients indicate that these cells produce IFN-γ; this cytokine production is believed to be associated with a protective response. Therefore, the major effector role of CD4 T cells in the response to *M. tuberculosis* is believed to be production of IFN-γ for activation of macrophages and subsequent destruction of intracellular bacilli. Studies of *M. tuberculosis*-infected CD4 T-cell-deficient mice did exhibit an early defect in overall IFN-γ production and macrophage activation (9), and this presumably was responsible for an early increase in bacterial numbers in the organs. However, as the infection progressed, other cells, most notably CD8 T cells, were also capable of producing this cytokine in the lungs, and overall IFN-γ levels increased to equal those of the wild-type mice. The CD4-T-cell deficient mice still succumbed to the infection, suggesting that early IFN-γ production by CD4 T cells was crucial to the control of the infection. However, a role for CD4 T cells apart from IFN-γ production was also suggested by those and other studies (6, 9).

In a persistent infection in mice, CD4 and CD8 T cells in the lungs produced IFN-γ (70; and J. L. Flynn and N. V. Serbina, unpublished data). Both cell types were found to be present in the granulomatous lesions in humans and mice (26, 31, 65). Depletion of CD4 T cells in a persistently infected mouse caused steady increases in the numbers of bacteria in all organs and in the death rates of the mice, demonstrating an essential role for these cells in control of persistent *M. tuberculosis* infection (70). However, examination of the mechanism by which these cells were participating in the prevention of reactivation revealed some surprises. Although CD4 T cells are believed to be major producers of IFN-γ in vivo, depletion of this subset did not result in an overall decrease in IFN-γ production in the lungs. The CD8 T-cell population produced more IFN-γ in the CD4 T-cell-depleted mice, as in the acute infection model. This CD8 T-cell-derived IFN-γ was sufficient to activate macrophages to produce RNI, as well. Therefore, the loss of CD4 T cells in the persistently infected mice did not lead to a deficiency in IFN-γ production or NOS2 induction overall. However, the mice were unable to control the infection, even in the face of wild-type levels of IFN-γ and NOS2. These data indicated that CD4 T cells have roles in the control of persistent *M. tuberculosis* infection that are independent of IFN-γ production and that RNI production by macrophages is insufficient to control a persistent infection. Other possibilities for the role of CD4 T cells in the control of *M. tuberculosis* include production of other cytokines, such as interleukin-2 (IL-2); effects on other cell populations, such as those of CD8 T cells or B cells; and other mechanisms of macrophage activation.

In contrast to the results of studies with the persistent-infection model, described above, depletion of CD4 T cells in the Cornell model of latent infection did not result in reactivation (76). In this same model, depletion of CD8 T cells had a detrimental effect on the ability of the mice to control the infection (76). Reconciling the differences between the two models and the effects of each T-cell population on the infection will be important to our understanding of protective immune responses during latent infection.

EVASION OF HOST IMMUNE RESPONSES

The establishment of a persistent infection demands that a microbe evade and subvert various immune mechanisms that

are meant to eliminate pathogens. In the case of latent tuberculosis, the host mounts a strong immune response that contains but does not eliminate the infection. Clearly the host response is effective in containment, since disruption of immune mechanisms can lead to reactivation of the quiescent infection, in humans and in animal models. However, the ability of the organism to survive in the face of a robust response clearly implicates a series of evasion mechanisms by the pathogen. Identifying these immune evasion strategies, as well as the mycobacterial genes involved, is central to our understanding of the pathogenesis of tuberculosis, as well as to the design of an effective vaccine.

M. tuberculosis-infected macrophages are rather ineffective at stimulating proliferation of and cytokine production by *Mycobacteria*-specific CD4 T cells (5, 35, 72). Infection of macrophages with *M. tuberculosis* can result in down-regulation of major histocompatibility complex class II expression or presentation (38, 47, 56, 62, 82). Other studies indicate that *M. tuberculosis* induces macrophages to produce immunosuppressive cytokines, such as IL-10 or tumor growth factor beta, and these cytokines impair the ability of infected macrophages to stimulate T cells effectively (36, 37, 68). There is also a recent report indicating that *M. tuberculosis*-infected macrophages are refractory to the effects of IFN-γ, a major mediator of macrophage activation (75). Thus, *M. tuberculosis* may modulate the macrophage in a variety of ways to prevent a strong T-cell response from recognizing and eliminating the intracellular bacilli.

MYCOBACTERIAL FACTORS IN LATENT INFECTION

The low tissue bacterial burden associated with tuberculous latency is a major obstacle to characterizing the mechanisms by which *M. tuberculosis* persists and reactivates in the host. This difficulty is further compounded by the lack of a much-needed genuine animal model of latent tuberculosis for stringently testing the validity of putative mechanisms underlying the persistence of the tubercle bacillus. Nevertheless, based on results of in vitro experimentation, various mycobacterial components have been identified as candidate persistent factors that may play a role in the establishment of the latent state of infection. More important, existing animal models, particularly those of the mouse, have been employed to evaluate the significance of these mycobacterial factors in tuberculous persistence. The use of the low-dose, persistent-infection murine model for tuberculosis, despite certain limitations, has been particularly useful for elucidating the roles of various *M. tuberculosis* components in tuberculous persistence. Examination of *M. tuberculosis* mutant strains deficient in some of the putative mycobacterial persistence factors in the chronic murine tuberculosis model has revealed that such mutants may display no apparent impairment in survival within the host; alternatively, they can be defective for growth or persistence. Mutants with growth impairment, compared to wild-type *M. tuberculosis*, fail to attain peak tissue bacillary burden during the initial rapid replicative phase in the host (16). Persistence mutants, while exhibiting no growth defect, cannot sustain the peak tissue bacillary load usually stably maintained by wild-type bacilli for a prolonged period of time (51). The implications of the in vivo phenotypes of deficiency in growth and persistence in the context of latent tuberculosis have yet to be defined. The distinction between gene functions in terms of growth and persistence is complex, and the two phenotypes may not be mutually exclusive. Here, we focus on mycobacterial components that have been shown to adversely affect the growth or persistence of the tubercle bacillus in the chronic murine tuberculosis model.

In vitro approach. The development of methods for the genetic manipulation of the tubercle bacillus (34, 63) and the recent availability of the entire *M. tuberculosis* genome sequence (13) have provided valuable tools for examining the mechanisms of tuberculous persistence. Exploiting these tools, various in vitro systems have been used to define *M. tuberculosis* components that may contribute to the establishment of persistence. The two most widely used in vitro systems for tuberculous latency are modeled, respectively, after anaerobic conditions and the stationary-growth phase, both of which are generally thought to be associated with the persistent state.

Using an in vitro anaerobic model of latent tuberculosis, Wayne characterized the growth of virulent *M. tuberculosis* in culture media during transition into a self-generated oxygen-depleted state (77, 78; see also reference 81, for a study by Wayne and Hayes). In addition to down-shifting to a nonreplicating dormant state thought to be akin to that in latent *M. tuberculosis* infection, bacilli in oxygen-depleted media were relatively resistant to antimycobacterial agents such as isoniazid but were susceptible to metronidazole, a drug effective against anaerobes (79, 81). Subsequent in vivo testing of metronidazole revealed, however, that this agent is not effective in the treatment of persistent murine tuberculosis (7, 19). This result raised questions concerning the degree of anaerobicity in persistent tuberculous lesion in the mouse (78, 81). The same in vitro model of tuberculous latency had been used to demonstrate the enhancement of the glyoxylate cycle, a metabolic pathway that facilitates bacteria to use acetate or fatty acids as the sole carbon source, in *M. tuberculosis* grown under anaerobic conditions (80). The activity of a key enzyme of the glyoxylate cycle, isocitrate lyase (Icl), has been shown to increase in long-term cultures of *M. tuberculosis* (55), and the production of this enzyme is enhanced under minimal growth conditions when the medium is supplemented with acetate or palmitate (39). Disruption of *icl* attenuated the ability of *M. tuberculosis* to persist in a murine model of infection; bacterial numbers of the *icl* mutant in the organs were lower than those of the wild type later in infection, although growth in the initial 2 weeks of infection was similar to that of wild-type *M. tuberculosis* (51). More important, the demonstration of a relative restoration of virulence of the *icl*-negative mutant in IFN-γ knockout mice suggests a link between the host immune status and the requirement for Icl (51). This observation implies that the host-bacterium interactions play an important role in the establishment of latent tuberculous infection.

M. tuberculosis sigma factors, because of their potential roles in regulating gene expression in response to environmental stress, have been targets of investigations in the context of virulence (reviewed in reference 29). A principal mycobacterial sigma factor, RpoV, has been shown to confer virulence, as assessed by tissue bacterial burden, to an attenuated *M. bovis* strain in a guinea pig tuberculosis model. RpoV was later shown to be the product of the primary mycobacterial sigma factor, *sigA* (29, 30). The *M. bovis* strain with attenuated viru-

lence harbors a missense mutation in its *rpoV* gene, resulting in an arginine to histidine change at position 522 (14). This R522H mutation is thought to impair the interaction of *sigA* with putative transcriptional factors essential for the expression of virulence genes (14, 30). A role for sigma factors in the persistence of the tubercle bacillus is further suggested by the recent demonstration of enhanced expression of *sigE* and *sigH* by *M. tuberculosis* during infection of human macrophages (32). The significance of these sigma factors in *M. tuberculosis* persistence in vivo remains to be determined.

Other *M. tuberculosis* products whose expression is not particularly related to anaerobicity and stationary-phase growth have also been identified as potential mycobacterial persistence factors. It is generally thought that intracellularly exported mycobacterial proteins are likely to contribute to inhibition of fusion of bacillus-containing phagosomes with lysosomes—a characteristic of *M. tuberculosis* that is potentially virulent. Based on this hypothesis, Berthet et al. have shown that exported repetitive protein, an intracellularly exported mycobacterial product, is essential for optimal growth in vivo (4). Directly examining the relationship of cell wall mycolic acids and virulence, Dubnau et al. have recently demonstrated that disruption of the *M. tuberculosis hma* gene results in defective oxygenated mycolic acid synthesis concomitant with deficiency in growth in the mouse (20). In evaluating the mechanisms for the association of cording and virulence, Glickman et al. have reported that *pcaA*, a gene that encodes mycolic acid proximal cyclopropanation activity and contributes to the serpentine colonial morphology of *M. tuberculosis*, is required for virulence and persistence in mice (28). Importantly, the lungs of mice infected with wild-type *M. tuberculosis* and *pcaA*-negative mutant revealed markedly different inflammatory reactions, suggesting that mycolic acid compositions may specifically modulate the host immune response to *M. tuberculosis*. These results, together with the observation that the virulence of Icl-deficient *M. tuberculosis* can be partially restored in IFN-γ knockout mice (51), underscore the importance of the complex interactions between the host and specific mycobacterial components in modulating the outcome of tuberculous infection. Finally, through studies designed to examine the effects of iron on the biology of the tubercle bacillus, mycobacterial factors that may contribute to persistence have been identified. Manabe et al. (46) used a dominant positive corynebacterial *dtxR* (diphtheria toxin repressor) to examine the role of iron in regulating the expression of virulence genes by *M. tuberculosis*. This iron-dependent repressor displays 80% identity in the functional domains with mycobacterial IdeR (iron-dependent repressor), and *M. tuberculosis* expressing the dominant-positive DtxR is defective for growth in the mouse.

In vivo approach. Adoption of signature-tagged mutagenesis (STM) technology has allowed an efficient means of direct in vivo identification of mycobacterial genes essential for survival in the host. In addition, the engineering of in vivo expression technology vectors has made possible in vivo trapping of promoters differentially expressed in the host during infection. Using STM technology, workers in two laboratories have independently identified mycobacterial genes that confer advantages for growth in the mouse (8, 16). Worthy of note, both groups have identified a region in the *M. tuberculosis* genome containing genes whose functions have been predicted to participate in the biosynthesis of phthiocerol dimycoserosate, a mycobacterial cell wall-associated complex lipid. Indeed, results obtained from biochemical analysis of transposon-mutagenized clones corresponding to this gene cluster have revealed that an intact *pps* promoter and *fadD28* are required for the synthesis of phthiocerol dimycoserosate, and *mmpL7* is responsible for transport of this *M. tuberculosis* lipid (8). STM technology thus provides a highly efficient method to systematically screen for genes critical for optimal growth in vivo. Exploiting the rapidly expanding database on antituberculous drug targets, an *inhA*-based in vivo expression technology system designed to screen for in vivo expressed mycobacterial genes is being studied. Preliminary results support the feasibility of this approach in trapping *M. tuberculosis* promoters that are preferentially activated in vivo (E. Dubnau, personal communication). Finally, research in another mycobacterial pathogen *M. marinum* has demonstrated the feasibility of the use of differential fluorescence induction, a fluorescence-activated cell sorter-based method to identify genes with enhanced expression in vivo (64). Mutation of two genes in *M. marinum* identified by this method, *mag* 24-1 and *mag* 24-3, results in deficient replication inside macrophages. These mutants also appear to be attenuated for their ability to persist in a frog granuloma model. The *mag* 24-1 and *mag* 24-3 genes are homologs of the *M. tuberculosis* PE-PGRS genes, predicted to encode a family of glycine-rich proteins of unknown function. It will be of interest to examine the role of PE-PGRS in the persistence of the tubercle bacillus in vivo.

Clearly, the use of the various in vitro and in vivo strategies described above has facilitated the identification of a rapidly growing list of mycobacterial factors that confer advantages for growth and/or persistence in the host. However, the significance of these factors in contributing to the ability of *M. tuberculosis* to establish latency remains unknown. Nonetheless, these approaches represent an important and significant first step toward the characterization of *M. tuberculosis* genes that might contribute directly or indirectly to the persistence of the tubercle bacillus in the host.

CONCLUSIONS AND FUTURE STUDIES

Latent *M. tuberculosis* infections present one of the major obstacles in gaining control over tuberculosis worldwide. Lack of information about the state of the bacillus during clinical latency hinders our ability to model latent tuberculosis in a laboratory setting. Animal models that truly represent human latent tuberculosis are also difficult to create and study. However, both in vitro and in vivo systems have been developed which contribute to our current understanding of latency. Host responses important in controlling the latent infection may include macrophage activation, maintenance of granuloma structure, CD4 T cells, CD8 T cells, IFN-γ, and TNF-α. Still to be tested are the contribution of other cytokines or chemokines and a multitude of other host factors to establishment and control of a latent tuberculous infection. The sequencing of the *M. tuberculosis* genome, as well as exciting new techniques for genetic manipulation and study of mycobacteria, are providing new avenues for understanding the changes the bacteria undergo to enter a persistent state and evade elimination by the robust response of the host.

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