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MYCOBACTERIAL PERSISTENCE AND IMMUNITY

Timo Ulrichs and Stefan H. E. Kaufmann

Department of Immunology, Max-Planck-Institute for Infection Biology, Schumannstraße 21/22, 10117 Berlin, Germany

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1. ABSTRACT

Tuberculosis still remains a major threat to global health at the beginning of the 21st century. The extraordinarily high numbers of morbidity and mortality are due to the ability of the pathogen, *Mycobacterium tuberculosis*, to evade an effective immune response and to persist within the host organism for long periods of time. The following article reviews our knowledge about both pathogen and host mechanisms involved in the immune response to *M. tuberculosis*.

2. INTRODUCTION

There are numerous reasons why tuberculosis still accounts for extraordinarily high rates of morbidity and mortality worldwide. Of prime importance is the ability of the etiologic agent, *Mycobacterium tuberculosis*, to persist for long periods of time in face of an active immune response and to adapt rapidly to the changing conditions inside and outside the host.

Tubercle bacilli are transmitted from patients with active disease by droplets which are then inhaled. After an incubation period of 4 to 12 weeks, only approximately one third of the individuals exposed become infected. Of these infected individuals, >90 % successfully contain the infection due to an efficient immune response. Replication and dissemination of the pathogen are restricted by mononuclear phagocytes; T cells are recruited to the site of primary infection containing the bacilli. In an attempt to avoid direct confrontation with the host immune defense, *M. tuberculosis* further retards its replication rate and transforms into a dormant [(a)] state. Thus, approximately one third of the world population, i.e. 2 billion people are latently infected with the pathogen. *M. tuberculosis*

remains dormant until the balance between bacillary persistence and the immune response gets disturbed. An impaired host response due to various reasons including aging, malnutrition, steroids or HIV allows reactivation of the bacilli resulting in clinical manifestation of tuberculosis (figure 1). Understanding the pathomechanisms of latent [(b)] persistence [(c)] of *M. tuberculosis* will therefore facilitate novel approaches towards prevention and control of infection, reactivation and reinfection.

Following inhalation of mycobacteria-loaded droplets, *M. tuberculosis* is engulfed by alveolar macrophages. The discovery that macrophages are involved in early mycobacterial infection in the lung dates back to 1893: "La cellule tuberculeuse est toujours une cellule lymphatique." (1). Further characterization of these infected macrophages in human and animal lung tissue revealed that acid fast bacilli were visible within the cells, though not cultivable (2, 3). It was unclear whether these "persistent" bacilli were in an altered developmental state and therefore not recognized by the immune system or whether most of the bacilli were no longer acid fast and therefore undetectable. Furthermore, chemotherapy seemed to reduce the risk of reactivation of remaining persistent bacilli (4). It was observed that drugs targeting metabolic functions of the pathogen such as isoniazid only act on metabolically active cells.

In order to study persistence of *M. tuberculosis* in greater depth, several models have been developed. So far none of them describes the mycobacterial infection of and persistence in the human host in satisfactory accuracy. McCune *et al.* developed a mouse model, in which tuberculosis-infected mice are treated chemotherapeutically until mycobacteria become undetectable by microscopic inspection or culture ("Cornell-model"; refs. 5 and 6). Following suppression of the host immune system by steroids, mice develop symptoms of endogenous reactivation of tuberculosis. *M. tuberculosis* must have survived in several organs in the infected animals and could restart replication (7, 8). However, the observed phenomenon of reactivation in infected mice is highly variable and the mouse models insufficiently mimick human *M. tuberculosis* infection and disease because mice do not develop progressive cavitating granulomas that are typical for human tuberculosis. Additionally, during chronic *M. tuberculosis*-infection in mice a high burden of bacilli is found within the organs whereas latent infection in humans is characterized by a low burden of bacteria which are rarely cultivable.

Reactivation is thought to develop mainly from the lung apices where latent bacilli are concealed (9). Primary lesions have been found to become sterile after several years. However, it remains unclear why lung apices - the preferred site for persistent mycobacteria - do not present typical granuloma formation consistently (10).

3. ENDOGENOUS EXACERBATION VERSUS EXOGENOUS REINFECTION

It is important to understand how, and to which extent, persistent *M. tuberculosis* infection is transformed into active disease at later time points, e.g. when the immune system is impaired. In a retrospective study in 1967, Stead *et al.* in investigating tuberculosis cases in post-war Europe realized that post-primary tuberculosis was primarily due to reactivation of latent mycobacteria (11). These results were confirmed by a study three years later (12), and the general view emerged that endogenous reactivation after latency is the main cause for active tuberculosis at least in industrialized nations. In 1971 - in the pre human immunodeficiency virus (HIV) era - an outbreak of multi-drug-resistant (MDR) active tuberculosis occurred among residents of a homeless shelter in Boston, and a subsequent investigation revealed that the MDR *M. tuberculosis* strain isolated from the patients caused exogenous reinfection in previously tuberculin-positive individuals (13). More recent studies investigated exogenous reinfection with MDR *M. tuberculosis* strains among HIV-positive patients using either phage-typing (14) or restriction-fragment-length-polymorphism (RFLP) DNA fingerprinting. In these studies high frequencies of exogenous reinfection rather than endogenous exacerbation were noted (15-17). These results were confirmed even for immunocompetent tuberculin-positive patients (18,19). An animal model aimed at distinguishing exogenous reinfection versus endogenous exacerbation provided controversial results: Experimental airborne reinfection of guinea pigs revealed that primary infection provided partial protection against dissemination of the bacilli suggesting improved immunity during later stages of infection and after granuloma formation (20). A mathematical model by Feng *et al.* incorporated the exogenous reinfection rate into their epidemiological model for the transmission dynamics of tuberculosis and concluded that exogenous reinfection played an important role in resurgence of tuberculosis in the past (21). A recent study documented a high incidence of tuberculosis among HIV-negative patients after exogenous reinfection by DNA fingerprinting in an endemic area of South Africa (22). The ongoing controversy about the relative contribution of both exogenous reinfection and endogenous exacerbation (23-25) highlights the importance to develop new (vaccination) strategies targeted against mycobacterial survival mechanisms during early, and especially late, stages of infection in order to control the disease and to prevent microbial dissemination.

4. MYCOBACTERIAL ENZYMES INVOLVED IN PERSISTENCE

In an attempt to mimick the environmental conditions within a lung granuloma, Wayne *et al.* established an *in-vitro* model of mycobacterial persistence (26,27). He proposed that *M. tuberculosis*, an aerobic acid-fast bacillus, adapts to the low oxygen content within the granuloma and thus fails to grow under normal culture conditions. This could explain the failure to culture "persistent" mycobacteria isolated from human lung granulomas. Wayne noted a metabolic downshift in mycobacteria during gradual oxygen-depletion (28). Further evaluation revealed that oxygen-deprived *M. tuberculosis* upregulates enzymes involved in the glyoxylate shunt, a metabolic pathway that converts fatty acids into carbohydrates. Isocitrate lyase

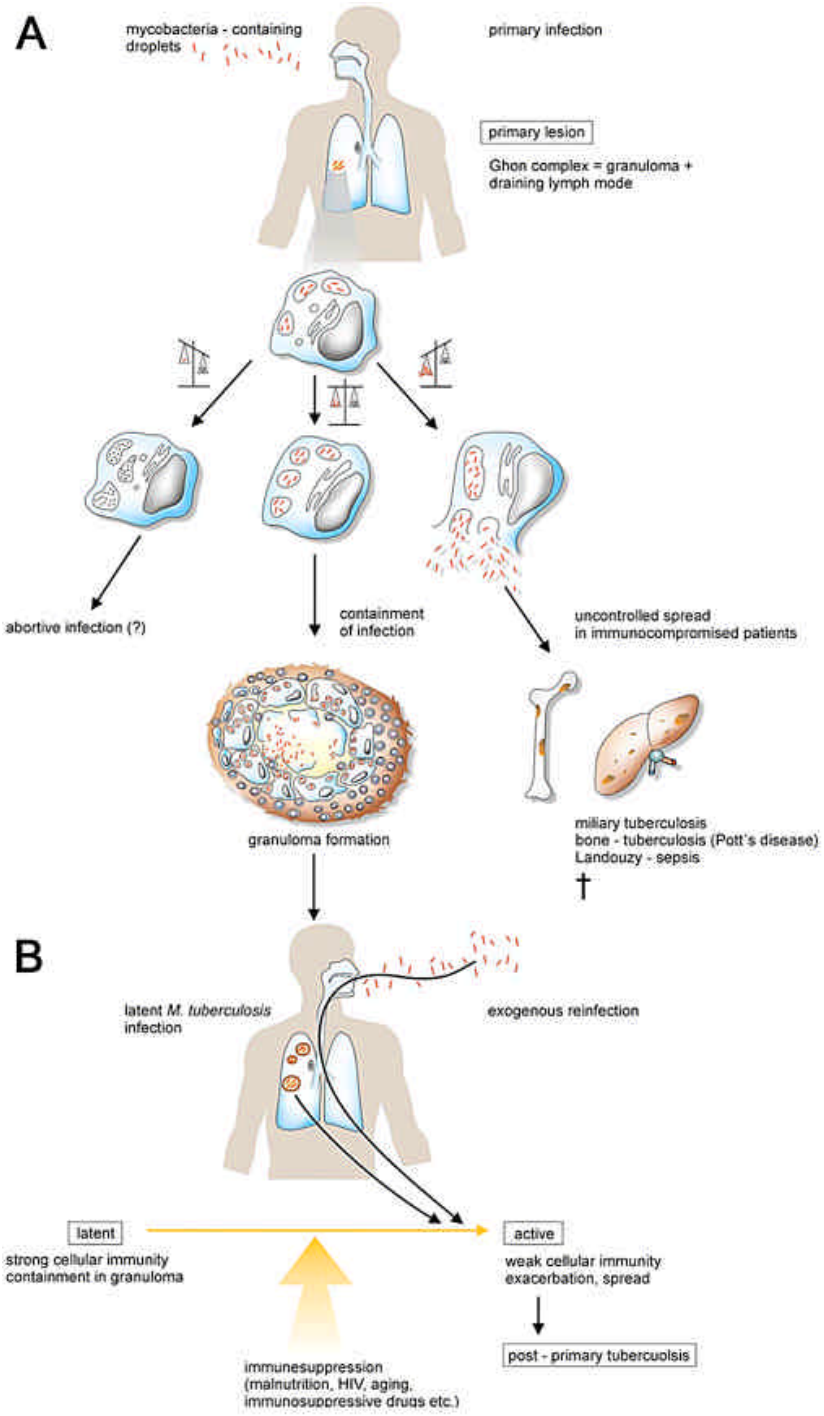

Figure 1. Balance between *M. tuberculosis* and the host immune system. A). *M. tuberculosis* is inhaled in droplets. After an incubation period of 4 to 12 weeks, infected alveolar macrophages containing the pathogen either destroy their predators (a mechanism that has not yet been proven, but probably accounts for a small proportion, left), or they fail to contain the pathogen and die (right). In the first case, infection is abortive, in the second case, the pathogen spreads throughout the body and causes active disease. Given that the immune response and virulence of *M. tuberculosis* are balanced (middle), intracellular bacteria are contained by the macrophages, and the immune system isolates the primary site of infection by granuloma formation (primary lesion). In this third scenario, infection without clinical disease develops. B). *M. tuberculosis* can persist in a dormant state for long periods of time. Any disturbance of the balance between host and pathogen after weakening of the cellular immune response (immunosuppression) causes endogenous exacerbation which leads to active (post primary) tuberculosis. Active tuberculosis can also be caused by exogenous reinfection.

and malate synthase convert acetyl-CoA derived from fatty acids into malate (via glyoxylate and via succinate), which can then be used for glucose synthesis (figure 2a and b).

The glyoxylate shunt allows *M. tuberculosis* to generate glucose independently from oxygen-consuming steps of conventional carbohydrate synthesis (26). A major advantage of the glyoxylate shunt is the usage of lipids as energy and metabolic source as they are abundant in the caseous center of the granuloma. More recent studies focused on isocitrate lyase as the key enzyme of the glyoxylate shunt and confirmed the findings of Wayne's *in vitro* model for the *in vivo* situation (29). McKinney *et al.* generated a *M. tuberculosis* mutant lacking isocitrate lyase and observed a declining numbers of mutant bacteria in infected mice in the later stage of infection (30). These findings suggest that the glyoxylate shunt is essential for the pathogen to survive in the developing granuloma and therefore essential for the adaptation to this altered environment in order to persist.

Adaptation of mycobacteria to the low-oxygen pressure within the granuloma also seems to require an anaerobic nitrate reductase that allows using nitrate as electron acceptor instead of normal oxygen respiration (31). A recent study investigated *Mycobacterium bovis* Bacille Calmette and Guerin (BCG) mutants lacking genes for the subunits of nitrate reductase and the observed decreased virulence in severe combined immune deficiency (SCID) mice, compared to wildtype *M. bovis* BCG (32). Adaptation to low oxygen pressure requires induction of various proteins, and only a small amount of them has been investigated thus far, e.g. an alpha-crystallin-homologue that is produced by *M. bovis* under O_2-deficient culture conditions (33,34). Glickman *et al.* generated a *M. tuberculosis* mutant deficient in cord formation due to malfunctioning cyclopropane synthase. This enzyme modifies mycolic acids by cyclopropanating the proximal end (35). The mycolic acids are components of trehalose-di-mycolate, the so-called cord-factor (figure 2c) and are major constituents of the mycobacterial cell wall. It has been suggested that this defect in mycolic acid synthesis alters the outer surface of *M. tuberculosis* affecting membrane fluidity, permeability and antigenicity. In the mouse model, the mutant bacilli replicated rapidly, but were unable to kill the host and decreased numerically at later stages of infection. Mutants which are unable to synthesize or to transport the cell-wall associated lipid phthiocerol dimycocerosate (PDIM) also fail to persist in the lung. PDIM is found only in cell walls of pathogenic mycobacteria and serves as virulence factor (36).

5. MYCOBACTERIAL GENE REGULATORS INVOLVED IN PERSISTENCE

The complete sequence of the *M. tuberculosis* genome provides the blue print for application of molecular-biological techniques towards elucidating gene regulation underlying mycobacterial persistence in the infected host (37). Numerous attempts have been made to investigate individual regulator genes involved in the mycobacterial response to changes in the host environment (reviewed in ref. 38). Amongst the first genes to be discovered were the genes encoding the RNA polymerase sigma factors which direct the transcription machinery to distinct genes required for bacterial survival under altered environmental conditions (refs 39,40 and figure 3a). One of the *M. tuberculosis* sigma factors, SigF (41), shows homology to a sigma factor of *Bacillus subtilis* which is involved in stress responses and in sporulation (42,43). The SigF is highly expressed during persistence, when replication and growth are downregulated, and is undetectable in the exponential growth phase, with a relatively high division rate (40). A *M. tuberculosis* mutant with an inactivated *sigF* gene failed to survive in later stages of infection when granuloma formation is induced (figure 1). The failure of the *sigF*-deletion mutant to adapt to the altered environment within the granuloma suggests its essential role for persistence of *M. tuberculosis* (44).

Another mechanism of microbial gene regulation plays a role in the adaptation of *M. tuberculosis* to nutrient starvation within granulomas: hyperphosphorylated guanine (p)ppGpp serves as gene regulator under starvation. The (p)ppGpp in *M. tuberculosis* is produced by the mycobacterial cognate of the Rel protein. A Rel-deficient mutant of *M. tuberculosis* showed reduced aerobic growth rate and impaired long-term survival under starvation and oxygen deprivation (45). Whether this mechanism of adaptation to starvation is also involved in the persistence of *M. tuberculosis* has to be further evaluated (figure 3b).

In the fast growing *Mycobacterium marinum* which causes tuberculosis in fish and amphibia differential gene regulation in granulomas and during exponential growth has been found (46). The genes identified by fluorescence induction and fluorescence-activated cell sorting (47) included two so-called PE-PGRS genes (figure 3c). The repeat PE-PGRS is shared by approximately 60 genes of *M. tuberculosis* which encode glycine-rich proteins with a characteristic glutamate-, proline-containing motif. Although the function of the PE-PGRS repeats remains unknown, directed mutations within the repeats impaired survival in granulomas (46). This approach could provide a tool to identify new mycobacterial genes involved in persistence. For example, genes belonging to the PPE family (with a proline-, proline- glutamate-rich motif) could be evaluated for their role in survival of *M. tuberculosis* in lesions. PPE and PE-PGRS genes belong to a larger family of PE genes, which account for approximately 10% of all encoding genes in the mycobacterial genome. Some of them have been recently identified by mRNA differential display to be only expressed in the virulent H37Rv strain and not in the avirulent H37Ra strain (48,49). Additionally, PE genes are upregulated in mycobacteria residing in granulomas and therefore could contribute to persistence (46).

6. HOST FACTORS INVOLVED IN PERSISTENCE AND LATENCY

An appropriate immune response can control mycobacterial growth (figure 4). Several studies have shown that the

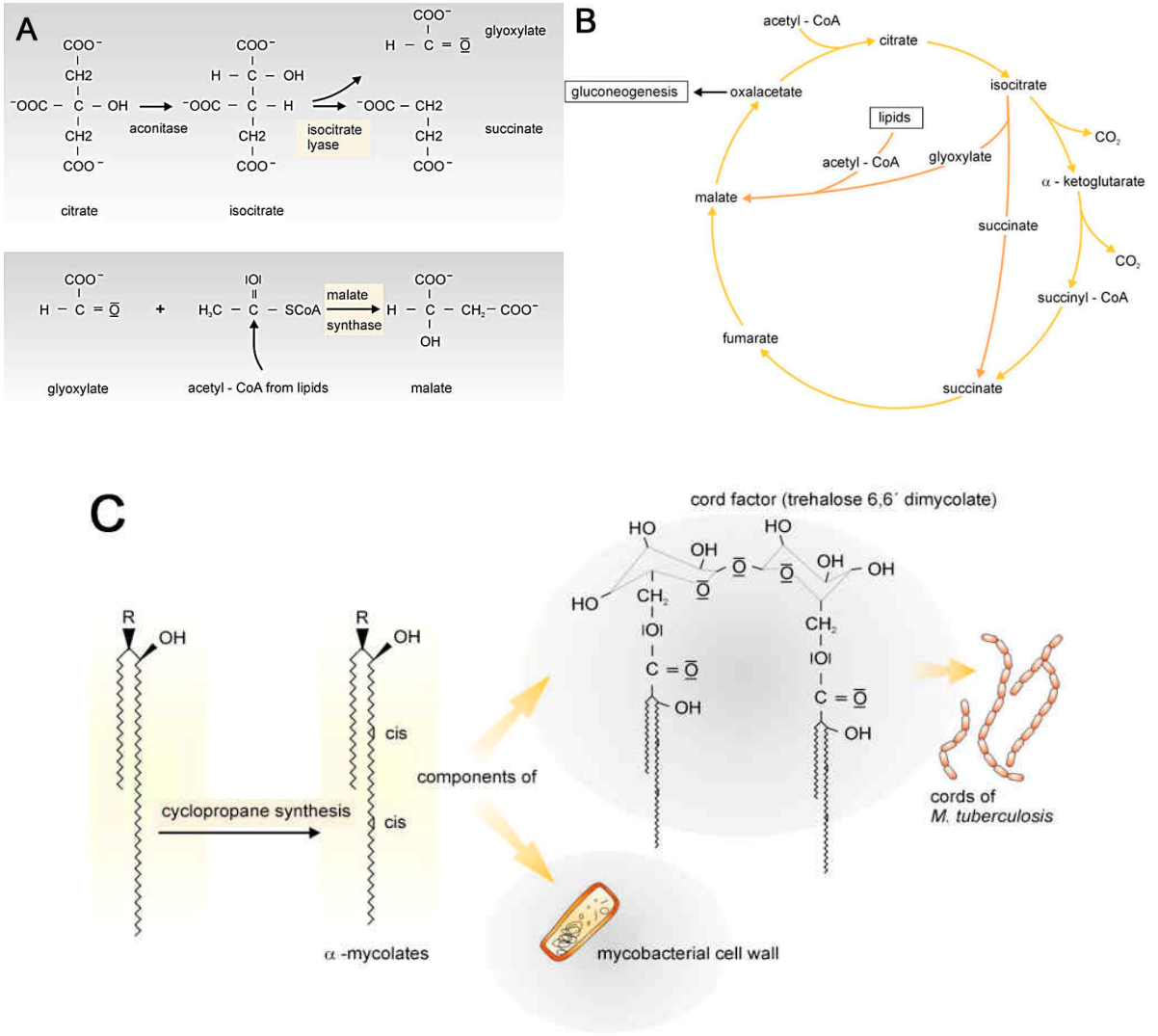

Figure 2. Glyoxylate shunt. The glyoxylate shunt allows *M. tuberculosis* to generate glucose independently from oxygen-consuming steps of the conventional synthesis of carbohydrates (citrate cycle). A. Aconitase transforms citrate into isocitrate. Isocitrate lyase catalyzes the cleavage into glyoxylate and succinate. Subsequently, glyoxylate can bind acetyl-CoA derived from lipid catabolism and is transformed into malate. B. Malate and succinate from this pathway can then be reintroduced into the citrate cycle. One major advantage of the glyoxylate shunt is the usage of lipids as energy and metabolic source which are abundant in the caseous center of granulomas. C. Cyclopropanase and mycolic acid synthesis. Cyclopropanase is required for modifying mycolic acids by cyclopropanating the proximal end. The mycolic acids are components of the trehalose-di-mycolate, the so-called cord-factor, a virulence factor which allows mycobacteria to form long cords.

cytokines interferon-gamma (IFN-gamma) and tumor necrosis factor- alpha (TNF-alpha) play a key role during the latent phase of infection. They activate macrophages to produce inducible nitric oxide synthase (iNOS) and to sustain pathways generating reactive nitrogen intermediates (RNI) (reviewed in refs.50 and 51). The TNF-alpha seems to play a role in containing persistent *M. tuberculosis* organisms and preventing them from reaching other regions of the lung or other organs (52,53). Encapsulation of the granuloma and formation of the fibrinous wall is primarily mediated by TNF-alpha (54). The TNF-alpha, given to mice depleted of CD4-positive T cells and latently infected with *M. bovis* BCG prevents recrudescence of infection (55,56). In a more recent study in mice, monoclonal antibodies against TNF-alpha caused an reactivation of latent *M. tuberculosis* infection. Interleukin-10 (IL-10) expression was augmented, IFN-gamma and IL12 p40 remained unchanged (57). This finding suggests that TNF-alpha prevents endogenous reactivation by modulating cytokine levels and limiting histopathology. Elevated levels of TNF-alpha, transforming growth factor-beta (TGF-beta) and IL-10 were also detected in sera and pleural fluids of patients with lung tuberculosis (58). In general, the risk of endogenous reactivation of latent *M. tuberculosis* seems to increase when normal levels of TNF-alpha are decreased. This has been emphasized by the fact that reactivation of tuberculosis represents a major side effect of anti-TNF-alpha-antibody therapy of severe rheumatoid arthritis (59,60).

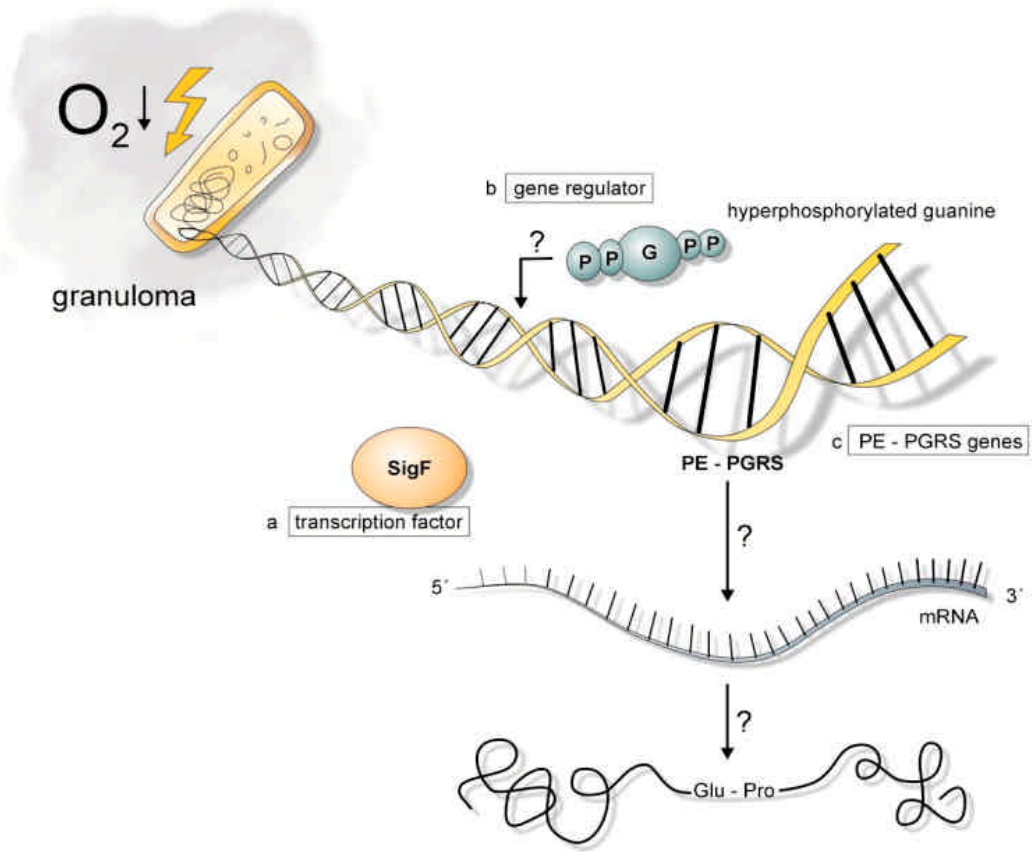

Figure 3. Gene regulation of *M. tuberculosis* within a granuloma. Several gene regulators are involved in mechanisms that allow *M. tuberculosis* to persist in granulomas under starvation and low-oxygen pressure. a). RNA polymerase sigma factors act as transcription factors. They direct the transcription machinery to distinct genes required for bacterial survival under altered conditions. One of them, SigF, is highly expressed in *M. tuberculosis* during persistence in granulomas. b). Hyperphosphorylated guanine (p)ppGpp serves as gene regulator under starvation conditions. (p)ppGpp in *M. tuberculosis* is produced by the mycobacterial version of the Rel protein, Rel(Mtb). c). PE-PGRS genes: The repeat PE-PGRS is shared by approximately 60 genes of *M. tuberculosis* which encode glycine-rich proteins with a characteristic glutamate-, proline-containing motif. The function of the PE-PGRS repeats remains unknown.

The IFN-gamma and iNOS (61) are also crucial for containing the infection and keeping the balance between replication of *M. tuberculosis* and immune defense (reviewed in ref. 51). Treatment with the iNOS inhibitor aminoguanidine impairs RNI production and causes reactivation of tuberculosis, leading to fatal disease in the mouse model (62). Although CD8-positive T cells participate in control of latent infection (63), substantial evidence emphasizes the major role of CD4-positive T cells in containing the disease at later stages: A recent study by Scanga *et al*. showed that CD4-positive T cells control persistent mycobacteria contained within a granuloma at least in part in an IFN-gamma and RNI-independent way (64). Depletion of CD4-positive T cells in the mouse model caused rapid reactivation of previously dormant *M. tuberculosis* organisms resulting in increased bacterial load and exacerbation to rapid-progressive tuberculosis. Endogenous reactivation of latent tuberculosis in the mouse model occurred despite normal levels of IFN-gamma and iNOS, suggesting that CD4-positive T cells regulate the balance between *M. tuberculosis* and activated immune cells. Both CD4-positive and CD8-positive T cells are found mainly in the periphery of intact granulomas, and their total number correlates with the structural integrity of the granuloma, underlining their key role in containing infection (figure 4). Production of lymphotoxin alpha3 (LT-alpha3) by CD4-positive T cells seems to mediate granuloma formation and maintenance: The LT-alpha3–deficient mice fail to form intact granulomas, and therefore suffer from exacerbated tuberculosis (65). Reduced numbers of CD4-positive T cells in HIV-positive patients are therefore a major risk factor for reactivation of persistent *M. tuberculosis*. Moreover, the lack of CD4-positive T cells for balancing persistent mycobacterial infection is a major cause of the recent resurgence of *M. tuberculosis* and the increase of active disease particularly in developing countries with high prevalence of HIV infection.

Animal and *in vitro* experiments cannot fully reveal host-pathogen interactions within the granuloma in the human lung. Immunohistological analysis of human

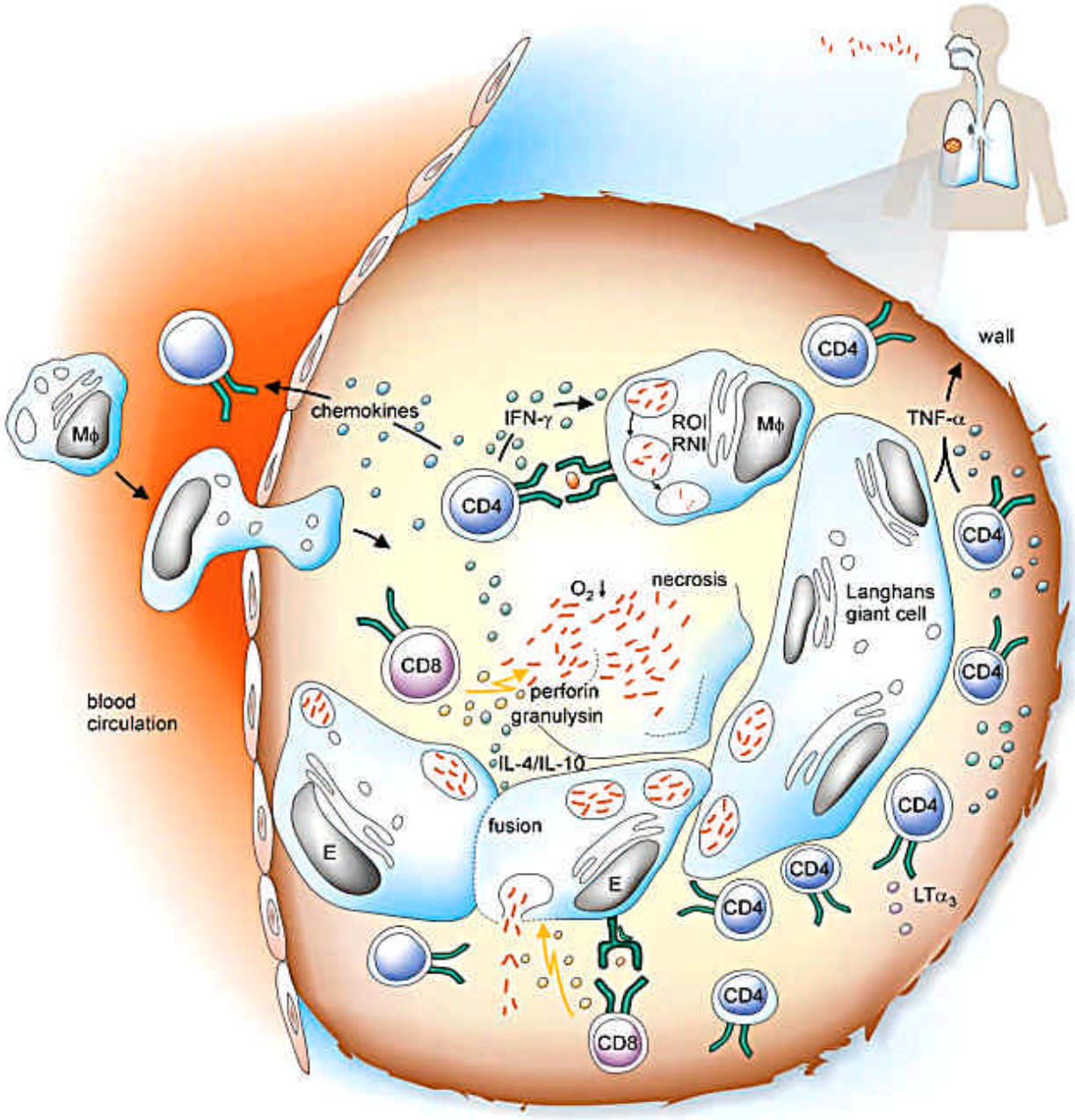

Figure 4. Host response and granuloma formation. The complex immune mechanisms that isolate the site of mycobacterial infection from the rest of the body still remain unknown. The following hypothetical picture can be drawn:Alveolar macrophages (Mϕ), epitheloid cells (E) or Langhans giant cells (generated by fusion of epitheloid cells) harboring intracellular mycobacteria form the center of the granuloma. They present antigens to T cells and activate them to produce a variety of cytokines and chemokines or to kill the infected cells and intracellular mycobacteria. Chemokines recruit additional cells from blood circulation to the site of primary infection. IFN-gamma activates macrophages and other antigen presenting cells to kill the intracellular bacteria via reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI). CD4-positive T cells produce TNF-alpha and lymphotoxin alpha 3 (LT alpha3) which are required for the formation of the wall surrounding the granuloma. In the center of the granuloma, cell detritus and low oxygen form a hostile environment for released mycobacteria. Activated CD8-positive T cells kill mycobacteria by means of granulysin and perforin. Killing of infected cells, however, needs to be controlled, in order to retain the integrity of the granuloma.

granuloma sections, staining and localization of mycobacterial antigens as well as isolation, expansion and characterization of T cells from human lung lesions will hopefully provide essential information about the balance between host and pathogen.

7. VACCINATION STRATEGIES AND MYCOBACTERIAL PERSISTENCE

Delineation of the *M. tuberculosis* genome and proteome offers new opportunities for better understanding issues of persistence and latency with direct relevance to vaccine design (37,66). Various strategies including subunit (67) and DNA (68) and live (69) vaccines can benefit from information arising from the genomic and postgenomic era. Live attenuated mycobacterial vaccines can be designed with the purpose of stimulating the immune system with immunodominant antigens as closely as possible to a natural infection with *M. tuberculosis*, but without the risk of persistence and recrudescence of tuberculosis at later time-points. *M. bovis* BCG was attenuated as a result of spontaneous deletions from the parental genome at the turn of the last century. Recent comparison with the *M. tuberculosis* genome revealed that together with deletions of virulence genes, genes encoding immunodominant antigens had also been lost (70,71). With the knowledge of the genes encoding regulators, enzymes and virulence factors important for persistence within granulomas and with the tools of gene replacement and transposon mutagenesis at hand (72,73), it has become possible to design a vaccine by targeted inactivation of genes encoding virulence factors responsible for persistence and exacerbation.

An alternative approach towards efficacious live vaccines is to improve the existing *M. bovis* BCG. This vaccine can be supplemented with genes encoding immunodominant antigenic determinants but devoid of harmful activity. Furthermore, *M. bovis* BCG can be improved in its immunogenic efficiacy, e.g. by enabling it to secrete cytokines activating the type 1 immune response (T-helper1 (T_H1) pathway; ref. 74), or to improve MHC class I antigen presentation (75). Both the attenuated *M. tuberculosis* and the improved *M. bovis* BCG need to be capable of persisting within the human host for a certain period of time in order to induce long lasting protection (76). Any risk of pathology or exacerbation of disease need to be excluded.

In the last years significant progress has been made in deciphering short and long term interactions between *M. tuberculosis* and the human host, and we are beginning to understand the mechanisms underlying potent long lasting immunity. Substantial evidence emphasizes that a T_H1 immune response is required, including IL-12/IL-18-dependent polarization of CD4-positive T cells and IFN-gamma activation of macrophages (reviewed in refs. 77,78). In murine models and more recently in the human system, the involvement of CD8-positive T cells has been demonstrated (reviewed in ref. 79). Furthermore, there is evidence that CD1-restricted T cells and gamma-delta T cells also participate in an appropriate immune response against *M. tuberculosis* (reviewed in refs. 80,81).

Many of the recent findings in the field of mycobacterial persistence that are presented above will be helpful in developing new strategies to keep *M. tuberculosis* in check:

Mycobacterial enzymes important for persistence in granulomas are potential targets for new drug developments that will pave the way for a better treatment, shorter regimens and the development of strategies to cope with MDR *M. tuberculosis* strains. Furthermore, these enzymes could be targets for new vaccine strategies.

The identification of **regulatory genes** like *sigF* or *relA* offers new possibilities to better understand the mechanisms of persistence and pathogenesis of *M. tuberculosis*. Delineation of the complete *M. tuberculosis* genome will further promote the identification of genes essential for persistence and latency.

Finally, the controversy about **endogenous exacerbation versus exogenous reactivation** stresses the need to identify genetic factors that predispose for endogenous exacerbation. In order to identify and better understand **host susceptibility factors** that promote transition from infection to active disease, a transcriptome approach will be best suited for evaluating global host profiles involved in controlling mycobacterial infection. Global evaluation of transcriptome profiles during infection may also answer the question why the immune response after natural infection with *M. tuberculosis* is insufficient and only provides incomplete protection against exogenous reinfection.

It is crucial that any new vaccine candidate elicits an immune response which comprises the appropriate combination of cells and factors necessary for optimum protection. Taking into account that one third of the world population is infected with *M. tuberculosis*, it is probably more feasible to develop a vaccine that promotes an active immune response which also prevents reactivation of dormant bacilli (figure 1) rather than focusing on a vaccine that prevents infection only. Obviously, it would be best if a single vaccine could be applied both as pre- and post-exposure vaccine. Since our knowledge about the strategies of mycobacterial persistence and the defense mechanisms and susceptibility factors of the host is still very limited, the development of a post-exposure or even therapeutic vaccine will be highly demanding. Since the time of Robert Koch who attempted to develop a therapeutic vaccine (tuberculin, or Koch´s remedy), various approaches have been undertaken to develop alternative strategies. The risk of side effects such as reactivation of latent bacilli by vaccinating a PPD-positive individual with BCG is generally contradictory. Therefore, any new approach has to be evaluated carefully. It is hoped that novel immunologic therapy such as subunit vaccination (67) or immunization using naked DNA (68) might circumvent these obstacles. Yet, even in these cases, a vaccine which only activates short term immune effector functions will be probably insufficient. Rather, a vaccine eliciting long term control mechanisms by the host immune system will be needed both for preventive and for therapeutic vaccines (82).

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Definitions:

[a] *Dormancy:* We define dormant *M. tuberculosis* as being in a state of low replication and strongly reduced metabolical activity.

[b] *Latency:* Although a technical term originally derived from virology, latent tuberculosis is used here to describe mycobacterial infection that does not present any clinical symptoms and is restricted to a contained primary site of infection harboring intact but dormant *M. tuberculosis*. The immune system fails to clear the mycobacteria from the organism.

[c] *Persistence:* Because this article focuses on immune mechanisms underlying control of tuberculosis, persistence is defined from an immunologic standpoint as survival in face of an ongoing immune response. Although we are aware that persistence can also be defined from a chemotherapeutic view we do not cover persistence of *M. tuberculosis* in presence of a chemotherapeutic agent aside from using the term "MDR" (multi-drug resistance). We assume that the reader can easily distinguish the obvious differences between the two types of persistence without additional explanations.

Key Words: *Mycobacterium tuberculosis*, Persistence, Latency, Vaccine, Review

Send Correspondence to: Dr. T. Ulrichs, Department of Immunology, Max-Planck-Institute for Infection Biology, Schumannstraße 21/22, 10117 Berlin Germany, Tel: (49[0]30) 28 46 0500, Fax (49[0]30) 28 46 0501, E-mail: ulrichs@mpiib-berlin.mpg.de