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PERSISTENT AND DORMANT TUBERCLE BACILLI AND LATENT TUBERCULOSIS

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1. ABSTRACT

Tubercle bacillus has remarkable ability to persist in the human host and has caused latent infection in one third of the world population. The current tuberculosis (TB) chemotherapy while effective in killing growing bacilli is largely ineffective in killing persistent or dormant bacilli, leading to prolonged therapy. There is considerable recent interest to study mechanisms of persistence and dormancy in mycobacteria. Meanwhile, there is also confusion about the use of terminology of dormant and persistent bacilli. Different models of mycobacterial persistence have been established. Various mycobacterial factors have recently been identified that may be involved in persistence or dormancy and resuscitation of dormant organisms. The phenotypic resistance to antituberculosis drugs in persistent and dormant bacilli presents a major challenge for effective control of the disease. The host immune system is critical in controlling latent TB infection from reactivation. A recent interesting observation is the reactivation of latent TB infection by anti-TNF-alpha antibody used as a treatment for rheumatoid arthritis and Crohn's disease. The role of psychoneuroendocrinological factors in TB, which is often ignored in the era of modern chemotherapy but could be important for controlling latent infection, is also briefly reviewed. There is recent interest to develop new TB drugs that target persistent and dormant bacilli and also immunotherapeutic agents that enhance chemotherapy and better control latent infection. The complex interaction between the bacteria, drugs, host and the environment underscores the need for a combined approach that incorporates chemotherapy, immunotherapeutic agents, improved socioeconomic, nutritional and even conducive psychological factors for more effective control of TB and latent TB.

2. INTRODUCTION

Tuberculosis (TB) remains a leading infectious killer worldwide, with 8 million new TB cases and 2 million deaths a year (1,2). The causative agent Mycobacterium tuberculosis is a remarkably successful pathogen that latently infects one third of the world population (1,2). The HIV infection, which weakens the

immune system and allows reactivation of latent TB infection, and the increasing emergence of multidrug-resistant TB (MDR-TB), pose a significant threat to the control of TB (1,2). Although the current TB treatment is "effective", it takes a lengthy period of at least 6 months (2). The reason for the lengthy therapy is thought to be due to the presence of persistent bacilli that cannot be killed by the current TB drugs, and that the host immune response does not appear to be sufficiently effective in ridding the host of the persistent bacilli.

Natural TB infection can become latent without any apparent symptoms that can last from a few weeks to a lifetime. Both bacterial and host factors come into play during latent infection, where the tubercle bacilli residing in the lesions in a quiescent state could reactivate when the host immune system is compromised. Dormant or persistent bacilli persist in the tissue despite immune response and chemotherapy. The ability of M. tuberculosis to persist in the host is primarily a feature of the tubercle bacillus itself largely independent of host immune response or drugs. It must be stated at the outset that the latency and dormancy phenomena are not unique to tubercle bacillus, as they also occur in other chronic and persistent infections such as streptococcal infections, syphilis, and H. pylori (3,4). The dormancy phenomenon is not even unique to bacteria. Plant, grass, and perhaps even tumor, have the ability to become dormant and reactivate or resuscitate later when the conditions are appropriate (5). Thus the ability to form dormant cells appears to be a property of all cells when there is a sufficiently large cell population, and certain factors such as starvation, aging, and low oxygen may facilitate the formation of dormant cells.

The topic of bacterial dormancy has received a lot of attention in recent years because of its medical and public health significance. Several reviews in this area are available (5-9). In addition, several reviews on mycobacterial latency have also been recently published (10-18). The purpose of this review is to mainly focus on M. tuberculosis with emphasis on new developments in mycobacterial dormancy and persistence and on host factors in the control of latent TB infection.

3. MYCOBACTERIAL DORMANCY AND PERSISTENCE

Interest in mycobacterial dormancy and persistence is primarily for two reasons. One is the frustration that the current TB drugs are unable to kill persistent or dormant tubercle bacilli leading to a lengthy chemotherapy, which frequently contributes to development of drug resistance due to poor compliance. The other reason is that tubercle bacilli have remarkable ability to persist *in vivo* and become dormant for years only to reactivate when the host immune response is compromised, thus posing a significant problem for effective TB control. Before moving onto the mycobacterial and host factors that are involved in persistence or dormancy, it is useful to define some of the terminology to avoid any confusion in this area.

3.1. Definitions of dormancy, persistence and latency

There is a great deal of confusion concerning the term dormancy, persistence, latency, and latent infection. The term dormant TB bacteria has been used without clearly or carefully defining its meaning. The imprecise use of the terminology has generated inconsistencies and confusion in this field. For example, bacilli in the Wayne model of "dormancy" have been called "dormant" bacteria when plenty of CFU-forming bacteria are present (19-21). To avoid any further confusion, the authors are encouraged to define the meaning of the terms when using the term dormant or persistent TB.

3.1.1 Definition of dormancy

Dormancy can be defined as a reversible state of bacterial metabolic shutdown (5,7). The term "dormant bacteria" refers to bacteria in a state of dormancy, where the bacteria with low metabolic activity remain viable but do not form colonies directly or immediately on solid medium but can be resuscitated to form colonies on plate under appropriate conditions (5,7). Important features of dormant bacteria include inability to grow directly on plates and resistance to various stresses and antimicrobial agents. The term "dormant bacteria" has been used carelessly by various workers and has created considerable confusion in the field. In many studies, "dormant" mycobacteria are not dormant at all as they are still CFU-forming (19-21). It must be emphasized that the definition of dormant bacteria requires resuscitation from non-CFU forming to CFU forming under appropriate conditions. Only recently the presence of dormant tubercle bacilli have been demonstrated *in vitro* in aged cultures (22-24), or in macrophages (25) using resuscitation procedures involving spent culture supernatant (22) or components derived from the supernatant (23,24). The "dormant" bacilli in all previous studies that did not involve the resuscitation may not be appropriately called dormant bacilli. The only exception is perhaps the persistent bacilli (may be called dormant in this case) in the Cornell mouse model after 3 month INH and PZA treatment (27), which did not form colonies on plates but caused relapse by immunosuppressive steroids. Dormancy phenomenon is not unique to M. tuberculosis, and various bacterial species also form dormant bacteria under appropriate conditions such as starvation, aging, low oxygen or low temperature (5-8). Although these are some of the conditions that can eventually induce the formation of dormant bacteria, the bacteria may not immediately become dormant under these conditions. The molecular basis of dormancy is not understood but its elucidation requires appropriate experimental models of dormancy (see below).

3.1.2. Definition of persistence

According to Walsh McDermott, a pioneer and expert in microbial persistence, the term persistence refers to "the phenomenon whereby otherwise drug-susceptible microorganisms have the capacity to survive indefinitely within mammalian tissues despite continued exposure to the appropriate drug or drugs" (27). McDermott and his colleagues tested 10 antituberculosis drugs singly or in combination as pairs of 2-3 drugs, which all failed to alter the persistence phenomenon. They went on, "Even the administration of pyrazinamide and a companion drug, a chemotherapy which rendered the tuberculous infection truly latent, i.e., hidden beyond the limits of diagnostic

reach, did not abolish the phenomenon of microbial persistence" (27). However, the above definition does not deal directly with whether the tubercle bacilli not killed by the drugs could form colonies on plate or grow in liquid medium. It can be inferred, however, that the bacilli in the persistence phenomenon could be CFU forming in the case of other drug combinations not containing PZA, or non-CFU forming in regimens that contain PZA, which could relapse spontaneously after cessation of therapy or induced with immunosuppressant steroids. Tubercle bacilli in the persistence phenomenon are often called persistent bacilli, which can be CFU forming or non-CFU forming. However, persistent bacilli cannot be equated with or called dormant bacilli. While the non-CFU forming persistent bacilli can be called dormant bacilli, the CFU-forming persistent bacilli in the persistence model cannot be called dormant bacilli. Here is a potential source of confusion and can create problem for people who study bacterial dormancy. In various studies with or without antibiotics, when the bacilli are not eliminated and remain at a low level in the host for a significant period of time, they were also called persistent bacilli, which are CFU-forming in this case. The distinction between persistent and dormant bacilli may be less important from the host point of view (since whether CFU-forming or not bacteria can cause reactivation of disease when the immune system is weakened). However, this distinction is important for the study of bacterial dormancy, because it deals with CFU-forming versus non-CFU forming bacteria, which are distinct entities that have different physiological basis in bacteriology.

3.1.3. Definition of latency.

To avoid confusion with the term dormancy, which is used to describe a bacterial property, the term latency should refer to *in vivo* situation where bacteria and the host have established a balanced state without causing apparent symptoms in the host, as in latent infection (28). Latent infection is commonly detected by tuberculin skin test (TST) and more recently also by detecting interferon-γ (QuantiFERON-TB test) produced by blood lymphocytes in response to specific M. tuberculosis antigens (29,30). It is worth noting that latent infection does not say anything about the metabolic or growth status of the tubercle bacilli in the host. It simply indicates the host is infected but has not developed symptoms. It could be that a small number of bacteria are actively multiplying but are controlled by the host so that an equilibrium is established between the bacteria and the host. Alternatively, it could also be that bacteria are just not growing or remain dormant in the host. Such distinction is important for understanding the role of INH in the prophylaxis of TB (see below). Latent TB infection can be treated with 6-9 months of INH, 4 months of RIF or 2 months of RIF and PZA (31,32).

INH has been used for prophylaxis to prevent recently infected individuals from developing into active TB (33-36). In the past, this use of INH has been called "preventative therapy" or "chemoprophylaxis" (34,35). To avoid confusion, the terminology has been recently changed to treatment of "latent TB infection" (LTBI) by an expert group organized by American Thoracic Society and CDC (31,32). However, the term LTBI is equally if not more confusing and misleading (a misnomer?) (28,37). The adoption of LTBI was perhaps also influenced by the use of INH to treat "inactive TB" (38). However, the author went back to the old paper and found that the term "inactive TB" really refers to a group of patients who had no active TB but with X-ray evidence of pulmonary disease very suggestive of TB and of longer duration without prior TB chemotherapy (38). It must be emphasized that tubercle bacilli in the "inactive TB" patients does not necessarily mean that the bacilli in the lesion are dormant or inactive. Similarly, LTBI does not necessarily indicate the residing bacteria *in vivo* are in a state of dormancy, since lack of apparent symptoms in the macro-organism (host) cannot be translated into lack of metabolic activity in the microorganism (bacteria) as being latent or dormant. INH is a drug that is active only against actively growing bacilli but not effective against non-growing bacilli (39). The proven usefulness of INH in the prophylaxis of TB strongly suggests that during LTBI there must be a small number of actively metabolizing bacilli that can be killed by INH. The LTBI during the early infection can be different from the latent infection by a small number of residual bacilli not eliminated by chemotherapy, which may not be growing and have slow metabolism. Yet, in both cases, the infected individuals appear healthy without apparent symptoms. However, INH while active against the small number of actively growing bacilli in the former case, would be expected to be totally ineffective against the small number of residual bacilli in the latter situation. Thus it is probably more appropriate to call the former case " recent or early TB infection" where there are actively growing bacteria rather than "latent TB infection", which gives the false impression that bacteria in LTBI are also latent or dormant, which is not the case. By avoiding the use of LTBI in the context of INH prophylaxis, it may help to avoid the peculiarity of INH being active against latent or dormant tubercle bacilli, as some would incorrectly assume.

3.2. Dormant and Persistent Bacteria as Part of Bacterial Life

Bacteria lead a life of “feast and famine”. We know quite well about how bacteria behave during growth phase, but know little about how bacteria behave during the latent phase of the bacterial growth cycle. We all know the typical growth curve has 4 phases, the lag phase, log phase, stationary phase, and death phase (40). The study of bacterial growth certainly appears to evolve in the same sequence of bacterial growth curve. When a bacterial inoculum is transferred to a fresh medium, growth does not occur immediately but only after a lag phase, bacteria start to replicate exponentially and bacteria in this phase are sensitive to antibiotics and stresses. When a batch culture (a closed system) is grown to stationary phase, bacterial growth halts because of exhaustion of essential nutrients and accumulation of toxic products where the bacteria become resistant to various stresses and antibiotics. Upon extended incubation in stationary phase, bacterial cells are beginning to die exponentially (death phase) (40), though nobody knows how they die. It is unclear whether the death phase is due to some active genetic program that triggers the death of bacteria or due to passive death as a result of lack of nutrients or starvation or toxic metabolic products

(9). It is increasingly appreciated that the bacterial cell populations upon entering stationary phase become more heterogeneous in morphology and metabolic states, while some populations die, others survive and remain viable and still others could even grow (cryptic growth) at the expense of dead cells (41-44).

The current bacterial viability (and of course the growth curve) is defined by culturability as in colony forming units (CFU) on plates or growth in liquid medium (5-7). The degree of bacterial viability depends on the time of incubation of a batch culture: the older the bacterial culture, the less viable bacterial cells. The number of CFU of an aged stationary batch culture is in general orders of magnitude less than the total number of bacterial cells in the culture (5). The difference in the total number of cells and CFU-forming viable cells, i.e., the nonculturable cells, could be due to dead cells or dormant cells. Whether a nonculturable cell is dormant or dead is not always easy to tell and has to be determined retrospectively by operationally or experimentally testing the bacteria under highly specific conditions that facilitate the resuscitation of the dormant cell. Kell and colleagues showed that an old (3-6 month) batch culture of the Gram-positive Micrococcus luteus while having very poor viability as judged by direct plating actually contained about 50% cells that were not dead and could be resuscitated to form colonies with a conditioned medium consisting of fresh medium and late log phase culture supernatant (45). Kell and co-workers identified a 16-17 kD protein secreted into the culture supernatant by Micrococcus luteus that has resuscitation activity and could increase the viability of the aged culture by about 100 fold (46). We have found similar resuscitation activity in the culture supernatant of M. tuberculosis (22). Therefore, it appears that bacteria can release autocrine factors into the culture supernatant that help to maintain viability. Besides resuscitation factors, bacteria also produce toxic or inhibitory factors into the culture supernatant, so that the viability of bacterial cells in a culture is controlled by two opposing activities much like Yin and Yang.

3.2.1. Formation of dormant bacteria

Not only the conditions that resuscitate the dormant bacteria are not clearly defined (see next section), but also the conditions under which the dormant cells form are not completely understood. Nutrient starvation, aging, low temperature, and oxygen depletion could induce or facilitate the formation of dormant cells in bacterial populations (22,24,45). Extended incubation or aging is often needed to facilitate the formation of dormant cells. It is not easy to obtain homogenous population of dormant bacteria without dead or live bacteria. More often than not, dormant cells are found in a mixture with dead and live cells in aged bacterial cultures. There is considerable debate as to whether dormant cells form through a pre-determined genetic developmental program akin to that in sporulating bacteria (8) or through a stochastic deterioration (47). Colwell and colleagues proposed that starvation and low temperature trigger a genetic program in Vibrio cholera that induces a viable but non-culturable state (VBNC) (8,48). The VBNC hypothesis has generated considerable debate about meaning of viability, dormancy and resuscitation. For a more detailed discussion of this topic please refer to references 5-8.

Although it is difficult to find dormant cells in young cultures, we have found that even young cultures contain dormant bacteria albeit in small numbers (Zhang Y, unpublished observations). It is well known that the number of viable bacteria (that can form CFU) even in young log phase cultures is always somewhat less than the total number of bacteria (49). For example, Wilson found that log phase E. coli always had less viable cells than the total number of cells, i.e. about 90% of the total number of bacteria (49). This in the past has been interpreted either as due to dead bacteria or to human errors. It is quite likely that this discrepancy could at least partly be due to the presence of small numbers of dormant bacteria that do not form CFU on plates. We have found that log phase cultures also contain dormant organisms, though in small numbers (Y. Zhang, unpublished observation). We hypothesize that all cultures contain dormant bacteria to some degree, even fresh stationary phase cultures, and the number of dormant cells increases as the culture ages.

3.2.2. Dormant bacteria and morphological changes

Bacteria undergo morphological changes, i.e., become smaller and coccoid or ovoid, upon extended starvation in old cultures (5,8, 50). While dormant bacteria may indeed have altered morphology, not all small and coccoid cells are dormant. Shleeva and colleagues obtained small ovoid coccoid tubercle bacilli in a 4 month old oxygen-starved culture with zero CFU (Shleeva *et al.*, 2002). Histological studies have indicated that tubercle bacilli in the lesions can have varying morphology, ranging from rod, ovoid, to granular shaped (51). The number of CFU formed from various types of lesions in many cases was considerably lower than the total bacterial counts (51). In some instances, tubercle bacilli resected from lesions in patients that had chemotherapy, could not grow initially but some of them showed growth only upon prolonged incubation of 9-12 weeks instead of 3-4 week usual incubation time (52). Yet in some other instances, tubercle bacilli are clearly visible in the lesions but failed to grow in normal culture medium (53-56). It is not clear whether these observable bacilli are dead or dormant. In addition, filterable ultra-fine tubercle bacilli, non acid-fast, also called, Much's granules or Much's bacillus originally discovered by Hans Much in 1908 (57), were often found in the tuberculous lesions (58-60) and from patients that had undergone TB chemotherapy (60). Inoculation of the guinea pigs with the ultra-fine forms did not cause typical TB but led to development of a peculiar granulomatous inflammation characterized by macrophages, mononuclear cells and epithelioid and giant cells (60). Cell wall deficient, non-acid-fast (NAF) tubercle bacilli could be formed *in vivo* in lesions or by treatment with cell wall hydrolase *in vitro* (61,62). Interestingly, the cell wall deficient forms were considerably less immunogenic (61) and thus may be responsible for the long term persistence without being cleared by the immune response. Do the coccoid forms revert to normal bacilli and cause disease? Some do and some do not (63). The idea of pleomorphic forms of bacteria as part of the bacterial life cycle (58,59,64,65) has been largely forgotten or ignored

by the mainstream bacteriologists but remains an interesting possibility that needs to be re-examined in the context of modern molecular biology and bacterial dormancy.

3.2.3. Location of dormant organisms *in vivo*

It is not clear where dormant bacilli are located *in vivo*, but it would be quite surprising if dormant bacilli should have a different location than non-dormant bacilli. Therefore, it is conceivable that dormant tubercle bacilli could reside both intracellularly inside host cells and extracellularly in the lesions. Interestingly a recent study in-situ PCR has shown that apparently normal lung tissue of patients without tuberculous lesions harbored tubercle bacilli in both macrophages as well as non-professional phagocytes such as type II pneumocytes, endothelial cells, and fibroblasts (66). TB DNA could also be detected by PCR in mouse spleen and lung tissues in the Cornell model (67). However, it is not clear whether the DNA is from dead bacilli or dormant bacilli in both studies. Reverse-transcription PCR (RT-PCR) is useful to determine if the bacilli are dormant or dead. Using RT-PCR, it was shown that the nonculturable or dormant tubercle bacilli in the Cornell mouse model had mRNA transcript for Antigen 85 (68), indicating that the dormant bacilli may still have some metabolic activity.

3.3. Models of Persistence

3.3.1. The mouse model of mycobacterial persistence

Although various circumstantial evidence suggests that M. tuberculosis can persist or remain dormant *in vivo* in humans for long periods of time (69), the most convincing evidence of dormant M. tuberculosis was demonstrated by McDermott and McCune and colleagues at Cornell University in New York in the 1950's and 1960's in a mouse model, called the Cornell model (70,71). In this model, mice were infected with virulent M. tuberculosis and the infection was allowed to establish for two weeks, followed by treatment with a combination of INH and PZA for 3 months. No bacilli could be demonstrated in the mouse spleen as assessed by plating tissue homogenates on agar medium after 3-month treatment with INH and PZA. However, one third of the mice relapsed with culture-positive tubercle bacilli when the treatment was discontinued for 3 months (70). Almost all mice relapsed with TB when immunosuppressive steroids were given (71). Clearly, dormant tubercle bacilli persisted in the tissue and were insensitive to antibiotic treatment. This indifference of the dormant bacilli to the drug treatment was not due to development of stable drug resistance, as these bacilli were still susceptible to the drugs upon subculture. This phenomenon of mycobacterial persistence is thought to be the cause for the lengthy TB chemotherapy. There are variations of the Cornell model that basically show the same disappearance and reappearance phenomenon of tubercle bacilli in mice with different infectious dose, route of infection (aerosol or intravenous), different drug combinations (72,73), and different agents other than steroids used in inducing the reactivation of the disease.

3.3.2. The Wayne model of TB "dormancy"

Because tubercle bacilli *in vivo* are thought to be located in low oxygen environment such as inside macrophages, granulomas or caseous lesions, Wayne established an "*in vitro* model of dormancy" where *in vitro* grown TB cultures are subjected to gradual oxygen depletion to mimic tubercle bacilli *in vivo* (74-76). Using this model of low oxygen tension, Wayne proposed a two stage nonreplicating persistence for M. tuberculosis *in vitro* (76). The first stage designated NRP 1 (nonreplicating persistence stage 1), occurred when the declining oxygen level reached 1% saturation (equivalent to microaerophilic conditions) and this stage is characterized by increased production of glycine dehydrogenase and steady ATP generation. The second stage, NRP 2, happened when the oxygen level reached 0.06% saturation (equivalent to anaerobic conditions) and this stage is characterized by a marked decline of glycine dehydrogenase and susceptibility to metronidazole (76). Nitrate reduction was increased in hypoxic shiftdown in non-replication persistence and was proposed as a marker for monitoring the shiftdown (77). The protein synthesis in the bacilli was shutdown in the Wayne model but remained responsive to heat shock and the bacterial viability remained unchanged (78,79). However, the bacilli in NRP 1 or NRP2 stage of the Wayne model were fully viable and gave about 10^8 cfu/ml (76), presumably because the relatively short time 10-14 days employed is not sufficient to convert most bacilli to dormant stage yet. (76). According to the definition of dormancy (which refers to bacteria unable to form colonies on direct plating but can be resuscitated under appropriate conditions), the Wayne TB "dormancy" model does not fit this criterion and appears to be more like a model of low oxygen adaptation. Nevertheless, the Wayne model has led to the identification of several factors such as isocitrate lyase (ICL) and glycine dehydrogenase that are related to persistence *in vivo* (80,81). Thus the Wayne model may represent a stage on the way towards dormancy and would still be a useful model to study dormancy as low oxygen may trigger dormancy upon extended incubation. Indeed, a recent study showed that longer incubation time of several months in the Wayne model appears to produce significant numbers of dormant bacilli (50). Despite the susceptibility to metronidazole by the bacilli under anaerobic condition in the Wayne model (76), metronidazole had little or no activity in the mouse model of persistent TB (82,83), indicating that the persistent bacilli *in vivo* may not be the same as those in the Wayne persistence model.

3.3.3. The rifampin “persister” model

This model was recently developed by Hu *et al.* (79), where a 100 day old stationary phase M. tuberculosis culture was subjected to incubation with high dose of rifampin (RIF) at 100 μg/ml for 5 days. The bacilli exposed to RIF treatment failed to form colonies on 7H11 agar plates but were able to grow in fresh 7H9 liquid medium. These authors went on to show the persistent bacilli still had the ability to metabolize C14-palmitate and produce mRNA for sigB, rpoB and hspX (79) The inability of the rifampin persisters to form colonies on plates but still grew in liquid medium is analogous to the dormant bacilli found in the Cornell model. A modification of the rifampin persister model is to add PZA along with RIF to old cultures, eliminating a further bacterial population and the residual bacilli more resemble dormant bacilli (79, see Mitchison review in this issue). The nonculturable or

persistent cells from RIF or PZA treated bacilli still had mRNA transcripts and incorporated radioactive uridine into their RNA, indicating that persistent bacilli still had transcriptional activity (79).

3.3.4. The resuscitation model

We have recently found resuscitation activity in the early stationary phase culture supernatant of M. tuberculosis that could resuscitate dormant bacilli in old batch cultures (22). We developed an *in vitro* dormancy model, where a liquid culture of M. tuberculosis is allowed to age for several months without shaking. Old cultures when plated out on plates formed few or no CFUs and the dormant bacilli in the old culture could be resuscitated with culture supernatant of M. tuberculosis or components derived from it to form more colonies on plates (22,23). Although CFU formation is the most reliable measure of viability, it takes lengthy incubation time especially for the slow growing M. tuberculosis. To circumvent this problem, we have used fluorescein diacetate/ethidium bromide double staining to rapidly monitor the resuscitation process (22).

3.4. Mechanisms of Dormancy and Resuscitation

3.4.1. Mycobacterial factors that may be involved in dormancy or persistence

It is quite likely that tubercle bacilli can actively manipulate the host immune system to its own advantage to allow for long term persistence in the host through secretion of bacterial factors or by changing its surface antigenic properties to avoid immune attack. However, these bacterial factors that modify the host immune response and the mechanism of mycobacterial persistence or dormancy are poorly understood, especially regarding how TB bacteria get into and maintain a dormant state or how persist in the host. Below is a list of mycobacterial factors identified using various approaches that may be involved in dormancy or persistence. However, it must be said that the role of many of these factors is putative and needs to be further assessed in a generally accepted model (such as the Cornell model) of dormancy and persistence.

3.4.1.1. SigF and SigB

In an attempt to identify mycobacterial sigma factors that might be involved in virulence and dormancy regulation, oligonucleotide primers were designed based on the conserved region of sigma 37 like sigma factors. PCR amplification revealed several PCR products from M. tuberculosis genomic DNA and partial sequencing analysis of one cloned PCR product revealed sequence homology to SigF of Streptomyces coelicolor (84). The complete M. tuberculosis sigF gene was obtained from a genomic library using the PCR product as a probe. The M. tuberculosis sigF forms an operon with an upstream gene usfX (85), encoding an anti-SigF (86). Interestingly, the inhibitory activity of UsfX can in turn be negatively regulated by two novel anti-anti-sigma factors, which appear to be regulated by redox potential and phosphorylation (86). SigF is present only in the slow growing mycobacteria and is induced in stationary phase and a variety of stress conditions such as nitrogen depletion, oxidative stress and cold shock (84), and anaerobic conditions and antibiotic exposure (87). Although there was no difference in *in vitro* growth and survival in macrophages compared with the parent strain, mutation in SigF caused reduced virulence in a mouse mode of TB infection (88). sigB was induced 2.5 fold under low aeration when the other sigma factor gene transcripts were lowered (89). sigJ was shown to be strongly up-regulated in the late stationary phase cultures (90), but further functional study to address the role of SigJ in the Wayne model is not available. However, none of the above sigma factors was induced in the Wayne model in a microarray study (91). It is unclear if there is any persistence or dormancy specific sigma factor or multiple sigma factors are required for dormancy formation.

3.4.1.2. ICL

Isocitrate lyase (ICL) is an essential enzyme for metabolism of fatty acids in the glyoxylate shunt pathway and was found to be induced earlier in the Wayne model (75). The icl gene is induced inside macrophages in tissue culture (92,93) as well as in macrophages in the non-necrotic lesion in human lungs but not expressed in the central necrotic lesion of granulomas in the lung (94). Its involvement in the persistence of M. tuberculosis in mice was initially identified by complementation of a M. smegmatis icl mutant with an M. tuberculosis genomic DNA library (80). ICL is not essential for the viability of tubercle bacilli in normal cultures or in hypoxic conditions, but important for long-term persistence in mice. Similarly, ICL from Candida albicans has also been shown to be important for persistence in a mouse model (95).

3.4.1.3. PcaA (cyclopropane synthetase)

PcaA was identified to be involved in persistence of M. tuberculosis in mice by a transposon mutagenesis approach based on changes in colony morphology and loss of cording (96). This gene pcaA (proximal cyclopropanation of alpha-mycolates) encodes a novel methyl transferase involved in the modification of mycolic acids in mycobacterial cell wall (96). PcaA knockout mutants grew normally *in vitro* and replicated in mice like the parent strain initially but were defective in persisting in mice. In the mouse survival experiment, mice infected with the PcaA mutant were still alive by 219 days whereas mice infected with the parent strain died (96). Several other cyclopropane synthetases such as CmaA1 and CmaA2 were also found in M. tuberculosis (97,98), but their role in persistence is not clear.

3.4.1.4. 16 kD-alpha crystallin (HspX, Acr)

The 16 kD alpha crystallin was originally identified as a 14 kD immunodominant protein antigen and was cloned by screening a lambda gt11 expression library using monoclonal antibodies (99). Sequence analysis revealed that the 14 kD or 16 kD protein is homologous to the small heat shock protein alpha crystallin (Acr) (100). Subsequent studies have shown that Acr is induced under a variety of conditions, such as by stationary phase (101), low oxygen conditions as in the Wayne model of dormancy (102-104), reactive nitrogen (105), and inside macrophages (106). The 16-kDa protein has been proposed to play a role in stabilizing cell structures during long-term survival in low oxygen conditions (102), and a marker of hypoxic

dormancy (91,107). Mutant of M. tuberculosis deficient in the 16 kD protein had decreased survival inside macrophages (103), though its role in persistence in mice remains to be determined.

3.4.1.5. RelA

Nutrient starvation induces stringent response in bacteria (108). In E. coli, the stringent response is a broad transcriptional program involving at least 80 genes (108). The stringent response is mediated by the signaling molecule hyperphosphorylated guanine (ppGpp) synthesized by RelA (ppGpp synthase I) and SpoT (ppGpp synthase II) (108). In M. tuberculosis however, there is only a single RelA homolog (98), which is a protein of 738 amino acids that is an ATP:GTP/GDP/ITP 3'-pyrophosphoryltransferase and an Mn^{2+}-dependent (p)ppGpp 3'-pyrophosphorylhydrolase (109). RelA mutation caused significant defect in long term survival *in vitro* and reduced ability to survive at anaerobic conditions, although the mutant appeared to behave as the parent strain in the initial growth phase and also survival inside macrophages (110). In a more recent study, it was shown that mice infected with RelA mutant had impaired ability to sustain chronic infection compared with the wild type strain H37Rv (Dahl *et al.*, 2003). In addition, significant histopathologic differences were noted in lungs and spleens of mice infected with RelA mutant compared with control strain H37Rv (111). Microarray analysis showed that the RelA mutant had a generalized alteration of the transcriptional apparatus, and also specific changes in the expression of virulence factors, cell-wall biosynthetic enzymes, heat shock proteins, and secreted antigens that may change immune recognition of the organism (111). These studies suggest that the M. tuberculosis RelA plays an important role in establishing persistent infection in mice.

3.4.1.6. PE-PGRS family

By examining mycobacterial genes that are preferentially expressed in macrophages in a frog granuloma model of tuberculosis caused by M. marinum, Ramakrishnan and colleagues found two homologs of the M. tuberculosis PE-PGRS family, that are involved in virulence and persistence (112). Mutation in the two PE-PGRS genes rendered M. marinum unable to replicate in macrophages with decreased persistence in granulomas (112). There are about 100 members of the PE-PGRS family in the M. tuberculosis genome with unknown biological function (98), but some of them have been shown to bind fibronectin (113) and be localized in the cell wall and cell membrane of M. tuberculosis (114,115). It has been proposed that the variable PE-PGRS proteins may be a form of antigenic variation that may provide an advantage for the bacilli to avoid detection and clearance by the immune system and allow long-term survival of tubercle bacilli *in vivo.* However, the role of PE-PGRS in persistence of M. tuberculosis *in vivo* needs to be more vigorously assessed.

3.4.1.7. Glycine dehydrogenase (GDH)

GDH encoded by gvcB, was induced during the early phase (NRP1) of adaptation of tubercle bacilli to anaerobic conditions in the Wayne dormancy model (75,117). Although the role of GDH in persistence of M. tuberculosis *in vivo* remains to be demonstrated, a recent study with Brucella abortus has found that GDH of B. abortus is required for the long term chronic persistence in a mouse model (81).

3.4.1.8. DevR-DevS/DosR-Rv3133

The two-component system DevR-DevS was initially identified as being preferentially expressed in virulent strain H37Rv over avirulent strain H37Ra in a subtractive hybridization analysis (118). In subsequent studies aimed at characterizing mycobacterial genes that are induced in the Wayne model, the same two-component response regulator pair was identified by microarray analysis and named Rv3133c/Rv3132c (91). Disruption of the Rv3134c located upstream of Rv3133c/Rv3132c eliminated the hypoxic regulation of alpha-crystallin acr (91), probably due to a polar effect on the downstream gene Rv3133c/DosR, which was later shown to be a transcription factor that controls the hypoxic related genes in tubercle bacilli, since inactivation of Rv3133c or DosR abolished the rapid induction of hypoxia induced gene expression (107,119). This suggests that DosR is a key regulator in the hypoxia-induced mycobacterial "dormancy" response (107). Although the mutant of DosR or Rv3133c grew as well as the wild type strain initially in a 5 day incubation, it survived significantly less well upon extended incubation up to 40 days in the Wayne model (107). However, conflicting data were obtained in a recent study involving DevR (DosR or Rv3133c) in SCID mice or immunocompetent DBA mice (120), where it was shown that DevR deletion caused "hyper-virulence" compared with the parent strain. This was demonstrated by reduced survival time for infected mice (30.5 days in DevR mutant infected mice versus 40.5 days for the parent strain infected mice), higher CFU in macrophages and infected organs, though the difference in bacterial numbers was modest, about 8-10 fold in organ CFU count and a 13% increase in the growth of devRΔ mutant in 24 h compared to a reduction of 50% in wild-type bacteria (120). The fast growing mycobacterium M. smegmatis has homologs of DevR/Rv3133c and Acr, and the same findings obtained for M. tuberculosis as above have also been found to be true for M. smegmatis (21,121). In addition, DevR or DosR mutant of M. smegmatis was not only more sensitive to hypoxia but also more sensitive to heat stress than the wild type bacteria (121).

3.4.1.9. NarX and NarK2, and NarG

In a comparative Northern analysis of 23 genes identified in M. tuberculosis genome that might play a role in energy metabolism under anaerobic conditions, Hutter and Dick found the mRNA level for narX, a putative 'fused nitrate reductase' absent from other bacteria, was strongly induced in anaerobic BCG bacilli (122). NarX was proposed as a possible useful marker for monitoring the persistent bacilli in infected animals. Using lacZ reporter promoter construct, narK2, encoding a putative nitrite extrusion protein, was identified that was induced in the hypoxic conditions in the Wayne "dormancy" model (123). M. bovis BCG lacking anaerobic nitrate reductase

(NarGHJI), an enzyme essential for nitrate respiration, failed to persist in the lungs, liver, and kidneys of mice (124).

In a whole genome microarray analysis, more than 100 genes whose expression is altered by hypoxia (0.2% oxygen) (91) were identified. Forty seven genes were induced including the 16 kD alpha-crystallin, NarX, NarK2 (nitrate extrusion protein), ferrodoxin (fdxA), bacterioferritin (bfrB), pfkB (phosphofructokinase II), ctpF (probable cation transport ATPase), glpQ1(phosphodiesterase), AhpC, and the response regulator Rv3132/Rv3133/Rv3134, and many other genes of unknown function. Several of the above genes [Rv0569, Rv2031c (HspX), Rv2623, Rv2626c, and Rv3841 (BfrB), Rv0363c (Fba) and Rv2780 (Ald)] have been shown to be induced in a S35-metabolic labeling experiment (125). Among about 60 genes that were repressed by hypoxic conditions, they were genes involved in protein, DNA, and lipid synthesis, amino acid and polyketide synthesis, and aerobic metabolism (91), indicating a reduced metabolic activity caused by hypoxic conditions.

Using a nutrient starvation model, Betts *et al.* characterized M. tuberculosis genes in a microarray and proteome analysis (126). Proteomic analysis of 6-week-starved M. tuberculosis cultures in a nutrient starvation model of mycobacterial persistence revealed the induction of several proteins (126), the 16 kD alpha-crystallin, a 24 kD hypothetical protein Rv2557, a 26 kD hypothetical protein Rv 2558. The 24 and 26 kD proteins are adjacent to each other in the genome and share 69% amino acid identity with unknown function. However, the expression of some proteins was decreased in this model, including the MPT64, and a chaperone protein homolog (Rv2462cTig), which is similar to B. subtilis trigger factor possibly involved in protein export. Microarray analysis indicated a general repression of transcription and metabolic slowdown in energy metabolism, lipid biosynthesis and cell division. On the other hand, the genes involved in stringent response and several other genes that may be important for long-term survival were induced in this model (126). It is worth noting that several sigma factors genes sigB, sigD, sigE, sigF and rsbW (anti-sigF) were induced but the RNA polymerase subunit genes rpoA and rpoC, were repressed (126).

3.4.2. Mechanism of resuscitation

How M. tuberculosis develops dormancy and how it exits dormancy into actively growing state is unknown and is a subject of current active investigation. We have recently identified a resuscitation activity from the culture supernatant of early stationary phase M. tuberculosis cultures, which could resuscitate dormant tubercle bacilli in old batch culture (22). In a subsequent study, the active components have been found to be phospholipids and an 8 kD secreted protein (Rv1174c) derived from the supernatant (23). Independently, Kell and colleagues have identified a 16 kD resuscitation factor from M. tuberculosis based on homology to Micrococcus luteus resuscitation factor (46) (see below). However, these studies are just a beginning and further studies are needed to understand the mechanisms of resuscitation.

It is well known that to start an M. tuberculosis culture requires a large inoculum size, and a small inoculum often fails to initiate the growth of M. tuberculosis in subculture (127). The reason behind this phenomenon is likely due to the requirement of a quorum sensing like growth enhancement factors. Culture supernatant of M. tuberculosis (22) and also active components derived from it such as phospholipids (23), could allow small inocula to initiate growth in liquid medium, which otherwise did not do so. The resuscitation phenomenon in M. tuberculosis only occurs with the culture supernatant of its own. Culture supernatants from other bacterial species such as M. smegmatis or E. coli had no effect on resuscitation of old dormant TB cells (22). It would be of interest to test whether the dormant tubercle bacilli in the spleen of mice, which fail to grow on agar plates as demonstrated in the Cornell model, could be resuscitated to form colonies on plates by the resuscitation activity of the supernatant. Indeed, preliminary studies indicated that some of the spleen tissue samples contain dormant bacilli that did not grow in normal culture medium such as 7H9 medium even upon extended incubation for 2-3 months, could be resuscitated and started growing in the presence of certain amount of spent culture supernatant (Y. Zhang, unpublished observation).

Another resuscitation factor, called resuscitation promoting factor (Rpf), which is a 16 kD protein derived from a Gram-positive coccus Micrococcus luteus, has been shown to stimulate the growth of not only itself but also a range of mycobacteria including M. tuberculosis (46). M. tuberculosis has five homologs of the M. luteus 16 kD Rpf in the genome (46). Subsequent studies have shown that the 5 M. tuberculosis Rpf when overexpressed and purified indeed had growth stimulating activity for M. tuberculosis (24). The 16 kD Rpf of M. tuberculosis (Musakulva and Kell 1998) could stimulate the growth of tubercle bacilli with a small inoculum prepared from old cultures, but did not stimulate the growth of actively growing BCG cells (24). The five rpf-like genes were expressed in actively growing cells, but not in non-growing cells (24). Antibodies raised against Rpf proteins could inhibit the growth of BCG *in vitro* (24). While the 16 kD Rpf is essential for M. luteus as it has only a single copy (24), the Rpfs in M. tuberculosis did not appear to be essential for viability as rpf knockout mutants are unaffected for growth presumably due to its redundancy in the genome (J. Chan and W.R. Jacobs, personal communications).

When discussing resuscitation of dormant bacteria, it is important to distinguish between resuscitation and regrowth. Resuscitation refers to dormant bacilli which otherwise fail to grow on plate or in liquid medium could regain this ability upon treatment with certain factors. However, regrowth refers to residual viable bacteria to start growth upon cultivation in common culture medium. These are very different concepts but in reality, unless a whole culture fail to form CFU due to formation of dormant bacteria, it is not always easy to distinguish the two. Indeed, in many instances both situations exist.

3.5. Phenotypic Resistance and Non-growing, Dormant or Persistent Bacteria

Antibiotics are usually active against growing bacteria but are ineffective against non-growing bacteria.

Hobby was the first to observe this phenomenon in 1942, when she found that penicillin, while killing replicating bacteria, was inactive against non-growing pneumococci in liquid cultures kept at 4 ^{o}C or in stationary phase (128). The reliance on bacterial growth for antibiotic activity was elegantly demonstrated by Kondo and Kanai using an ingenious approach involving a streptomycin-dependent M. tuberculosis strain. They showed that the effectiveness of chemotherapy is closely associated with bacterial growth in a mouse model (129). In the absence of streptomycin where there was no growth of tubercle bacilli, the TB chemotherapy was not effective in killing the bacilli but was effective in the presence of streptomycin where the streptomycin-dependent bacilli could replicate. This study indicates that bacterial replication is necessary for the drugs to kill the bacteria. The insusceptibility of the non-growing bacteria to antibiotics is due to changes in bacterial metabolism or physiological state and is thus called phenotypic resistance. There are at least three types of phenotypic resistance based on their physiological state. They are, stationary phase phenotypic resistance, persister phenotypic resistance, and phenotypic resistance in dormant bacteria. When bacteria are not growing as in stationary phase or in biofilm infections associated with implanted device, they are not susceptible to antibiotics even though the bacteria are viable (130-133). In E. coli the phenotypic resistance to antibiotics and biocides was shown to be dependent on the expression of stationary phase general stress response sigma factor RpoS induced by ppGpp (134). Thus it is likely that the phenotypic resistance to antibiotics is part of a general stress response in non-growing cells.

Another type of phenotypic resistance or drug tolerance relates to the phenomenon of “persisters”, which are best shown by survivors after antibiotic exposure in liquid cultures. When a log phase culture containing a large number of bacteria is exposed to antibiotics, there are always a few residual persisters that are not killed (3,135). Although the mechanism is not well understood, it has been proposed that the persisters may have different transcription profile compared with the majority of the bacterial population, providing the persisters an advantage to survive upon antibiotic exposure (136). In a recent study, it was shown in an E. coli system that mutation in LexA3, an enzyme involved in DNA repair caused increased susceptibility to different antibiotics compared with lexA wild type strain (137), suggesting that DNA repair is important for the survival of persisters to antibiotic exposure. It is unclear if the “persisters” not killed by antibiotics in culture underlie the mycobacterial persistence *in vivo.* That dormant tubercle bacilli are insusceptible to TB drugs is best demonstrated in the Cornell mouse model where despite extensive chemotherapy for several months the dormant or persistent bacteria remain viable and can cause reactivation when the host immune system is compromised by steroids (26). It is likely that all three different types of phenotypic resistance may share some common mechanism, however, this topic is not well understood. The mechanism of phenotypic resistance in M. tuberculosis is unknown and is an important topic for future study. The phenotypic resistance is a major problem for antibiotic therapy, especially for the TB therapy.

Non-growing bacteria can also roughly be divided into two different types depending on their ability to grow immediately upon subculture into a defined fresh medium. Stationary phase bacteria and the "persisters" not killed by antibiotics will grow upon subculture into fresh medium whereas dormant bacteria will not grow unless appropriate resuscitation factors are present, reflecting different metabolic status of the non-growing bacteria. The non-growing forms are a constant source of confusion for people who study bacterial persistence or dormancy. This confusion largely stems from the fact that very little is known about bacterial life styles, especially the non-growing bacteria, the definition of dormancy and that of viability. It also stems partly from careless use of the term without defining it.

The current theory of TB treatment is the "special bacterial population" hypothesis of Mitchison who proposes that the tubercle bacilli in pulmonary lesions are not homogeneous, but consist of heterogeneous bacterial populations (138). According to this hypothesis, INH kills actively growing organisms (Population A) when the TB strain is susceptible to INH, but if a strain is resistant to INH, this population will be killed by streptomycin, ethambutol and rifampicin. Rifampicin also kills a population of slow metabolizing bacilli (Population B). PZA, on the other hand, kills a population of semi-dormant non-replicating bacilli residing in acid environment in the lesion (Population C). It is noteworthy that no TB drugs are effective against the dormant bacterial population (Population D). The different bacterial populations with varying degree of metabolism (from high to low) underlie the difficulty in achieving complete eradication of TB and the need for prolonged 6 month TB therapy. It is worth noting that the inability of chemotherapy to completely eliminate tubercle bacilli in the lesions is not because drugs do not reach the bacilli in the lesions, in fact they do (51). While the mechanism underlying the above lengthy TB chemotherapy is not clear, it is widely believed that the prolonged treatment (6 months) required to cure TB is due to the presence of a population of persistent bacilli with low metabolism in the tissue that are in a physiologically distinct state such that they are not susceptible to the available TB drugs. The slowness of the therapy is also determined by the nature of the tissue lesions characteristic of the disease. For example, high numbers of bacilli (as high as 10^{8-9} bacilli) in cavities, or small number of bacilli in solid caseous lesions where there is no apparent growth of bacilli due to lack of oxygen, pose problems for effective killing by TB drugs (51). Although active pulmonary TB cases are rendered noninfectious by the standard TB chemotherapy in a few weeks (139), the remainder of the lengthy therapy is still needed to eliminate a population of persistent bacilli ("persisters") in the lesions to a low enough number while allowing the host immune response and local tissue defense mechanism to develop and control the residual bacilli not killed by TB chemotherapy. It is likely that understanding the mechanism and control of mycobacterial persistence and dormancy may have implications for designing new antituberculous drugs, which can further shorten the lengthy 6 month TB therapy through affecting the persistent or dormant tubercle bacilli.

In a recent study, Wallis and colleagues found that different clinical isolates of M. tuberculosis had a differing ability to withstand the killing effect of antibiotics, a term the authors called drug tolerance (140). The tolerance is phenotypic as these isolates were apparently susceptible to antibiotics by the conventional drug susceptibility testing (140). Tolerance to INH and ethambutol correlated with tolerance to rifampin, and interestingly, the drug tolerant isolates appeared to be associated with prolonged persistence or relapse, suggesting a nonspecific mechanism (140). It remains to be seen if drug tolerance might affect the outcome of TB therapy in a more systematic study.

4. HOST FACTORS AND LATENT INFECTION

Various clinical observations and experimental studies suggest that host factors play an important role in the control of latent infection caused by tubercle bacillus. Among individuals that are infected with M. tuberculosis, only about 10 percent will go on to develop clinical disease. The remainder 90% of infected individuals has latent infection without apparent symptoms for months or years or even lifetime. Any factors that disturb the fine balance between tubercle bacilli and the host can lead to reactivation of disease. Such factors can be extreme physical and psychological stress, malnutrition, use of immunosuppressive agents (steroids), or infection with another agent such as measles or HIV, or even other bacterial infection (strep) (141), or other predisposing disease conditions such as diabetes and silicosis. Many of these factors are not well defined in terms of how they allow latent infection to become overt disease and thus are not always easy to control. These factors not only trigger reactivation of disease but can also make the individuals more susceptible to new infection. Other factors such as bacterial virulence, inoculum size, host genetic factors may also potentially influence the outcome of latent infection.

The current 6 month TB therapy, also called "DOTS" (directly observed treatment, short-course), is the best therapy against TB and can achieve up to 95% cure rate (2). Although TB patients are rendered non-infectious after the first few weeks of chemotherapy (139), the remainder of the 6 month therapy is necessary to kill a population of slowly-metabolizing persistent bacilli and to allow the host to develop sufficient immunity to keep at bay the small number of residual bacilli not killed by the TB drugs. Thus antituberculous therapy cannot achieve a complete bacteriological cure, and the host immune response plays an important role in achieving a clinical cure of the disease. The fact that the TB chemotherapy takes so long (6 months) is telling evidence that the human host or the TB drugs are not very effective in getting rid of persistent bacilli. Yet, it is interesting to note that a significant proportion of TB patients were cured with 3-4 months therapy with no relapse (142-144). While it is possible that the disease in those that were cured may be less severe and had no unfavorable drug metabolism, the difference in cure rate could reflect differences in host factors such as immune response and nutrition status that influence the relapse. Detailed analysis of the patients that have relapse in terms of their immune function and nutritional status etc will be useful to understand factors that control latent TB infection. One can never be sure that a cured patient will not relapse during lifetime and the persistent bacilli have completely lost the ability to reactivate. This kind of nagging uncertainty is partly a reflection of our incomplete understanding of persistence phenomenon and partly a reflection of on-going dynamic interaction between the host and bacteria inherent to the latent TB infection.

It is not easy to tell whether the TB bacteria in latent infection are multiplying or simply remain dormant in latently infected individuals with no apparent symptoms. It is quite likely that both types of bacilli are present in latently infected individuals within different types of lesions. Yet, it may be useful to distinguish new infection (i.e. recent skin test conversion) and inactive infection resulting from previously "cured" disease. Distinguishing the above possibilities may have practical implications for INH prophylaxis for latent infection and for addressing possible spread of TB from apparently "healthy" but latently infected people. INH prophylaxis has been shown to be useful for preventing development of disease in recent latently infected individuals as well as for preventing reactivation of "inactive" latent infection in high risk individuals (old people) who had TB in the past (38). This appears to suggest that tubercle bacilli are multiplying at least sporadically in latently infected individual, and if host immune response fails to hold the bacilli in check or no INH prophylaxis is given, the latently infected individual may go on to develop TB or relapse.

Two murine models are available to study the role of host factors in latent TB infection. One is a low dose infection model in a relatively resistant mouse strain such as C57BL/6 mice characterized by low and stable bacterial counts without death of animals where the small number of tubercle bacilli is kept under control by host immune response with minimal disease (145-147). This model is characterized by granulomas and small patches of alveolitis, with high expression of TNF-alpha, inducible nitric oxide synthase (iNOS), and Th1 cytokines IL-2, and IFN-gamma (147). The latency established by the small number of bacilli can be disrupted leading to reactivation of disease by administration of steroid in both Bcg^s and Bcg^r mice (148). The second model is the Cornell model (26) (see above section). Various manipulations such as neutralization of cytokines with antibodies or cytokine knockout can be used to assess their role in controlling latent infection and preventing reactivation in the two models. In humans, however, the above manipulations cannot be used and thus the various factors involved in controlling latent infection are more difficult to assess. Recently, a nonhuman primate model using cynomolgus macaques was developed that mimics the latent human infection, where 17 macaques were infected with a low dose of virulent M. tuberculosis via bronchoscopic instillation into the lung and the outcome of infection was monitored (149). About half of the monkeys developed active TB whereas the other half had latent infection with no apparent disease. The authors suggested that the low-

dose infection of cynomolgus macaques appears to mimic the full spectrum of human M. tuberculosis infection (149). This model may provide a useful tool to address the various immune factors that control latent TB infection.

Molecular fingerprint analysis can be used to address if the disease is due to reactivation of latent infection or reinfection. In a recent study, it was shown that an apparent reactivation of latent TB infection in a son 33 years after his father suffered from TB, who both harbored the same TB strain, was identified using IS6110 fingerprinting analysis (150).

4.1. Immune Factors

The appropriate host immune response that control latent TB infection is not well understood. However, recent developments in this area shed some new light on the role of immune system in controlling latent infection. In a murine model of latent infection with BCG, anti-T cell monoclonal antibody besides steroids and cyclosporin A caused reactivation of disease with increase bacterial load in the spleens, indicating that the bacterial counts during chronic BCG infection are maintained by discrete T cells (151). In a recent study in mice, CD4+ T cells were found to be important in controlling latent TB infection, since depletion of CD4+ T cells by antibody caused reactivation of disease, with increased CFU in the infected organs and more severe pathology in the lungs and reduced survival (152). However, in another study using anti-CD4 and anti-CD8 antibodies (153), it was found that CD4+ T cells were important for controlling acute phase of infection in a mouse model but had little effect on the latent phase of infection. In contrast, CD8+ T cells while not active in acute phase of infection, played a more significant role in latent phase of infection (153). Reactivation of the latent infection through activation of the hypothalamic-pituitary-adrenal (HPA) axis, is associated with a switch from Th1 cytokines to Th2 cytokines, whereas control of latent infection is associated with Th1 type of cytokines attributable to phenotypically different CD4 and CD8 populations (154). M. tuberculosis may use various strategies to evade the immune system, however, the effector molecules and detailed mechanisms are not well understood. Macrophages infected by M. tuberculosis are less effective in stimulating the proliferation and cytokine secretion of specific CD4+ T cells, through downregulation of MHC class II expression and antigen presentation (155-158). Secretion of immunosuppressive cytokines such as TGF-beta (159) and IL10 (160) by macrophages may be one of the mechanisms in attenuating the immune response to M. tuberculosis infection and may be involved in reactivation of disease (161). In a recent study, TGF-beta was found to suppress the immune response by causing increased apoptosis in M. bovis activated CD4+ T cells (162). In a mouse model of Leishmania major persistence in the skin after healing in resistant mice C57BL/6, CD4+CD25+ regulatory T cells accumulated in the dermis were involved in maintaining the persistence by suppressing effector T cells by IL-10 dependent and IL-10 independent mechanisms (163). It will be of interest to assess the role of CD4+CD25+ regulatory T cells in persistence of TB infection.

Tumor necrosis factor (TNF)-alpha plays an important role in protection and immunopathology of TB (164-166). Neutralization of TNF caused slightly increased bacterial load, intense inflammation and destructive tissue pathology with loss of granuloma structure (167), and also reactivation of latent TB infection in a proportion of mice (72). Interestingly, Infliximab (Remicade), a humanized anti-TNF-alpha antibody used for the treatment of a range of inflammatory diseases such as rheumatoid arthritis (168) and Crohn's disease, could induce reactivation of latent TB causing active disease in patients (169-173). These clinical studies clearly demonstrate that TNF-alpha plays a crucial role in keeping latent TB infection in check without reactivation under normal conditions. It is quite likely that some chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease may actually be beneficial in terms of preventing latent TB infection from reactivation due to increased TNF-alpha production. INF-γ is involved in host resistance to mycobacterial infection due to its ability to activate macrophages and induction of INOS (14). However, neutralizing INF-γ did not appear to induce reactivation of latent TB infection in the Cornell model (72,153). Reactive nitrogen intermediate (RNI) is important for killing tubercle bacilli (174). Aminoguanidine, an inhibitor of nitric oxide synthase involved in production of RNI, could cause reactivation of TB in a murine low dose latent infection model or a drug-induced latent infection model (175,176). The reactivation was accompanied by enlarged spleen and liver, a more severe tissue granulomatous reaction, and increased CFU in the infected organs (175). The role of innate immunity such as antimicrobial peptides, chemokines, complement and Toll-like receptors in controlling latent infection remains to be determined.

4.2. Psychoneuroendocrine Factors and Other Disease Conditions

Psychological stress has long been known to play a certain role in the onset of tuberculosis (177-179). There were numerous examples where intensive physical or emotional stress could lead to reactivation of latent TB infection resulting in active disease. As Dubos clearly showed in his book The White Plague, TB incidence rocketed during the War times, a remarkable demonstration on the role of psychological stress in development of TB presumably due to reactivation of latent TB (180). Another close example is that Dubos's first wife Marie Louise Dubos developed reactivation TB during the Second World War and later died of TB, apparently because of extreme emotional anxiety and worries for her family in France, which was then at war with Germany (181). The topic of psychological factors in the development of TB was much talked about prior to the development of antituberculosis chemotherapy (177-179). Unfortunately, with the availability of TB chemotherapy, this topic seems to be largely forgotten in the Western world, with the exception of some Eastern European countries such as Russia and Poland (182-184), as if psychological factors no longer play any role in susceptibility to infection and in control of latent TB infection, which is not true. Nutritional factors also influence the susceptibility to TB by affecting the host defense mechanisms (185), however, in real world TB

control this aspect is rarely taken into consideration. There is no doubt that chemotherapy plays a very important role in TB control. However, the limitation of sole reliance on chemotherapy for TB control is becoming increasingly apparent. One wonders if chemotherapy is so wonderful and effective, why do we still have such a high incidence and prevalence of TB worldwide? TB was predicted to be on its way out even before the advent of chemotherapy at the beginning of last century (180), yet with the availability of effective chemotherapy TB incidence though decreased, is still high today. The reason is that chemotherapy deals with only one aspect or step of the TB problem that is determined by many other factors such as negative socioeconomic conditions, emotional stress, malnutrition and other disease conditions such as HIV infection that are independent of TB chemotherapy. As Dubos put it, TB is a social disease of incomplete civilization (180). A multifactorial approach is needed besides chemotherapy to more effectively control TB.

It is well known that psychological factors or stress can influence the immune response causing susceptibility to a range of diseases including infectious diseases through the neuroendocrine hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes, resulting in the dysregulation of the immune system (186,187). However, the role of psychological stress and neuroendocrine factors in the control of latent TB infection has not been studied but will be an interesting area of future investigation.

Although use of glucocorticoids in conjunction with TB chemotherapy to reduce the inflammation and effusion can be beneficial for the treatment of certain forms of TB such as tuberculous pleurisy (188,189), meningitis and percarditis (190), glucocoticoids use can suppress the immune response and cause increased susceptibility to TB and in the absence of TB chemotherapy could lead to reactivation of latent TB as in the treatment of systemic lupus erythematosus (SLE) patients (191,192). In a mouse model of TB, antiglucocorticoids dehydroepiandrosterone (DHEA) and its derivative, 3beta,17beta androstenediol (AED), particularly AED, were protective, which caused a decrease in bacterial load, enhanced granuloma formation and prolonged survival (193). The protective role of AED correlated with the appearance of cellular infiltrates rich in cells expressing IL-2, IL-1alpha and TNF-alpha, and partial suppression of the switch to IL-4 producing cells (193). This study suggests a potential therapeutic role of AED in the treatment of TB.

It is well known that diabetes is a high risk factor for development of TB (194) and causes frequent relapse of TB in diabetics (195,196). In diabetes, there is significant endocrine disorder such as alteration in thyroid and gonadal function, in addition to elevated levels of glucocorticoid levels (197-199), which could lead to suppression of the immune system and cause increased susceptibility to TB and its reactivation. It is worth noting that the production of IL-1 beta, TNF alpha and IL-6 in diabetic TB patients was significantly lower than that in the TB patients with no diabetes (200). Since IL1 and TNF-alpha are associated with resistance and protection against M. tuberculosis infection in mice (201), it is likely that decreased production of these two cytokines in diabetic patients could lead to increased susceptibility and reactivation of TB.

Silicosis of the lung is another risk factor for TB, which can persist for a life time. The TB chemotherapy is often difficult to achieve a complete cure of TB in such patients (202). Because silicosis makes the host more susceptible to TB, silicotic guinea pigs could be used to demonstrate the presence of potentially dormant bacilli from tissues which otherwise fail to grow in culture or cause TB in healthy guinea pigs (203). The underlying mechanism of increased susceptibility to TB and the basis for the difficulty in completely eliminating TB in silicosis is not well understood. Loading of alveolar macrophages with silica dust could impair the function of macrophages, resulting in reduced ability to phagocytose and kill tubercle bacilli. Silica has been shown to potentiate the growth of M. tuberculosis in macrophages (204). Recently, it has been shown that polymorphisms in the regulatory elements of proinflammatory cytokine TNF-alpha and IL-1 are associated with silicosis (205).

5. STRATEGIES TO COMBAT PERSISTENT AND DORMANT BACILLI

The current TB treatment relies on the chemotherapy, but this approach has its limitation as the current drugs are not effective in eliminating dormant or persistent bacilli. Developing new and unconventional drugs that kill the persisters or dormant bacilli and enhancing the immune system may be needed for more effective control of the disease.

5.1. Development of Drugs against Dormant or Persistent Organisms

Current TB drugs are mainly active against growing bacilli but are not active against non-growing persistent bacilli, except for RIF and PZA (133,138). These two agents are important sterilizing drugs that significantly reduce the number of bacilli in infected tissues and shorten the therapy from previously 12-18 months to 6 months (138,206). However, persistent bacterial populations that are not killed by the TB drugs still remain, which poses significant problem for a more effective control (133, Mitchison, this issue). Currently, there is renewed interest to develop new drugs that target the dormant and persistent tubercle bacilli with the hope to shorten the therapy. Strategies aimed at taking advantage of unique physiology of tubercle bacillus such as deficient efflux of pyrazinoic acid (207) are worth exploring. For example, we have recently found that M. tuberculosis seems to be uniquely susceptible to weak acids (208) based on our study on the mode of action of PZA (209, see Wade and Zhang, this issue), and weak acids could be developed into potential new drugs that target persistent bacilli. Factors that are likely involved in persistence as reviewed above, such as ICL (80), cyclopropane synthases PcaA (96), could be attractive targets for drug development. The crystal structure of ICL (210) and PcaA (211) are already available. ICL is being actively pursued as a target of drug

design (210). Although several ICL inhibitors were identified (80), the results on their use *in vivo* in mice for eliminating persisting or dormant bacilli are not yet available. In addition, identification of the genes that are switched on during resuscitation process, genes involved in starvation survival (126) or general stress response (212) or genes involved in Wayne persistence model (91) could also be potential new drug targets for developing drugs against persistent and dormant bacilli. For a more detailed discussion on this topic, please see reviews by Zhang and Amzel (133), Coates *et al.* (213) and Mitchison (this issue). Besides trying to kill dormant or persistent bacilli, another approach would be to "wake up" the dormant bacilli by resuscitation factors so that they become susceptible to antibiotics. Although various resuscitation factors have been identified (23,24), the effect of this approach on eliminating dormant or persistent organisms *in vivo* remains to be tested in animal models of TB infection.

5.2. Immunotherapeutic Agents

In addition to developing drugs that kill the dormant bacilli, another approach to control the dormant or persistent bacilli is through activation of appropriate immune response to prevent reactivation of latent infection. Although this is a potentially important and conceptually attractive approach for more effective TB control, in the absence of adequate understanding of the protective immunity to TB infection, no immunotherapeutic agent that can achieve this goal has been developed so far. In one study, vaccination with hsp60 DNA was shown to prevent the relapse of latent TB infection in the mouse model (214). However, another study by Repique *et al.* did not find significant effect of DNA vaccination (Esat-6, Ag85B, KatG, MTB39A (PPE), Rv1818 (PE), along with 5 other MTB genes encoding culture filtrate proteins) in preventing the reactivation of TB infection induced by steroid dexamethasone (215). It is unclear if the discrepancy represents differences in different antigens tested or methodology. In addition, a recent study by Taylor and colleagues showed that vaccination with hsp60 and Ag85 DNA caused adverse effects in mice previously exposed to TB (216), raising some concern about the safety of DNA vaccination in individuals who may have already been exposed to M. tuberculosis. Since non-growing persistent bacilli are thought to be the cause for the inability of TB drugs to eliminate the bacilli, it was reasoned that use of immunosuppressive steroids could allow the bacilli to start growing, which would make the bacilli susceptible to TB drugs and lead to more effective treatment (51). However, in a clinical study, steroids failed to provide any benefit for improving the treatment (217). M. vaccae , a fast growing soil mycobacterium, could potentially serve as an immunotherapeutic agent (218, and Stanford *et al.*, this issue). Although use of M. vaccae or BCG did not have significant effect on the relapse of TB in the Cornell mouse model (219), M. vaccae used in conjunction with DOTS chemotherapy appeared to shorten the TB therapy in patients presumably by enhancing the immune system to more effectively kill persistent bacilli in a preliminary study (220, Stanford *et al.*, this issue). Further studies with more patients are needed to assess the role of M. vaccae as an adjunct of TB chemotherapy for improved treatment. Cytokines such as IL-2, IFN-gamma, IL-12, and GM-CSF, which are involved in protective immunity (221), remain to be tested for their effect on controlling dormant or persistent organisms and shortening the TB treatment.

6. CONCLUDING REMARKS

Tubercle bacillus is a remarkable pathogen that can persist in the host for long periods of time despite immunity. Knowledge of bacterial and host factors involved in TB persistence and dormancy is still meager despite some recent progress in this area. There is a great need to study the host-mycobacteria interaction, not just in the sense of how mycobacteria cause disease (virulence properties), but also how mycobacteria manipulate the host immune system to its advantage to survive and cause latent infection, and how the host cells respond to mycobacterial infection, and how such knowledge can be translated into better control of persistent and dormant TB bacteria and latent infection. There is also a need to develop more sensitive techniques to analyze dormant or persistent bacteria in tissues and to apply the microarray technology to the study of bacterial genes and host genes involved in dormancy and latency.

Already one third of the world population is latently infected with tubercle bacilli. How to prevent latent infection from reactivating and devise strategy to kill dormant or persistent bacilli represents a major intellectual and practical challenge for a more effective TB control. What are the factors that allow only about 5-10 percent of those latently infected individual to go on to develop disease? An equally important or perhaps more important question is why majority of latently infected individuals do not get disease. The current TB control is not optimal as it relies primarily on chemotherapy, which can only achieve clinical cure but not bacteriological cure. Nevertheless, it may be useful to implement a (targeted?) mass INH prophylaxis in endemic areas with high TB incidence to prevent new latent infection from developing into active disease. While there is little doubt that development of new drugs that kill persistent or dormant organisms is very likely to achieve a shorter course of therapy, in final analysis, adequate host response is required to prevent relapse of treated patients and prevent latent infection from developing into overt disease. If we cannot completely eliminate the dormant organisms or persisters in the body, can we manipulate host factors to achieve a balance between pathogen and immune response and prevent latent infection from becoming overt disease? What defines a healthy and appropriate host response that control latent infection? How can we tap the psychoneuroendocrine system to control the latent infection? Answers to these questions are likely to lead to improved disease control. To emphasize the importance of these factors that impact host resistance to TB infection, Rene Dubos said in his classic book "The White Plague" that TB is a social disease of incomplete civilization (180). Eradication or control of TB cannot be achieved by chemotherapy alone but is influenced by many factors such as socioeconomic, physical and psychological factors. This disease outlook would entail a new therapy where simultaneously attacking

the pathogen by chemotherapy and enhancing the host immune response, psychoneuroendocrine system and nutrient status can be harnessed to benefit the host and better control TB and latent TB infection.

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8. REFERENCES

1. Dye, C., S. Scheele, P. Dolin, V. Pathania & M. C. Raviglione: Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *JAMA* 282, 677-686 (1999)
2. The World Health Organization Global Tuberculosis Program. http://www.who.int/gtb/ (last accessed 6/03)
3. McDermott, W.: Microbial persistence. *The Yale J. Biol. Med*. 30, 257-291 (1958)
4. Rhen, M., S. Eriksson, M. Clements, S. Bergstrom & S. J. Normark: The basis of persistent bacterial infections. *Trends Microbiol.* 11, 80-86 (2003)
5. Kell, D.B., A. S. Kaprelyants, D. H., Weichart, C. L. Harwood & M. R. Barer: Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. *Antonie Van Leeuwenhoek* 73, 169-187 (1998)
6. Kell, D. B. & M. Young: Bacterial dormancy and culturability: the role of autocrine growth factors. *Curr. Opin. Microbiol.* 3, 238-243 (2000)
7. Barer, M. R. & C. R. Harwood: Bacterial viability and culturability. *Adv. Microb. Physiol*. 41, 93-137 (1999)
8. Colwell R. R..: Bacterial death revisited, in *Nonculturable Microorganisms in the Environment.* Ed., Rita R. Colwell and D. Jay Grimes, p. 325-342, ASM Press, Washington, D.C. (2000)
9. Nystrom, T.: Not quite dead enough: on bacterial life, culturability, senescence, and death. *Arch. Microbiol*. 176, 159-164 (2001)
10. Wayne, L. G.: Dormancy of Mycobacterium tuberculosis and latency of disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 13, 908-914 (1994).
11. Wayne, L. G. & C. D. Sohaskey: Nonreplicating persistence of *Mycobacterium tuberculosis*. *Annu. Rev. Microbiol.* 55, 139-163 (2001)
12. Parrish, N. M., J. D. Dick & W. R. Bishai: Mechanisms of latency in *Mycobacterium tuberculosis*. *Trends Microbiol*. 6, 107-112 (1998)
13. Honer zu Bentrup, K. & D. G. Russell: Mycobacterial persistence: adaptation to a changing environment. *Trends Microbiol.* 9, 597-605 (2001)
14. Flynn, J. L. & J. Chan: Tuberculosis: latency and reactivation. *Infect. Immun.* 69, 4195-4201 (2001)
15. Ulrichs, T. & S. H. Kaufmann: Mycobacterial persistence and immunity. *Front. Biosci.* 7, d458-69 (2002)
16. Riska, P. F. & S. Carleton. Latent tuberculosis: models, mechanisms, and novel prospects for eradication. *Semin. Pediatr. Infect. Dis.* 13, 263-272 (2002)
17. Zahrt, T. C.: Molecular mechanisms regulating persistent *Mycobacterium tuberculosis* infection. *Microbes Infect.* 5, 159-167 (2003)
18. Gomez, J. E. & J. D. McKinney: Persistence and Drug Tolerance, in TUBERCULOSIS, 2nd ed, Ed., William N. Rom and Stuart Garay, Chapter 8, p. 101-114, Lippincott Williams & Wilkins, A Wolters Kluwer Company, New York (2003)
19. Wayne, L. G. & H. A. Sramek: Metronidazole is bactericidal to dormant cells of *Mycobacterium tuberculosis. Antimicrob. Agents Chemother.* 38, 2054-2058 (1994)
20. Murugasu-Oei, B & T. Dick: Bactericidal activity of nitrofurans against growing and dormant *Mycobacterium bovis* BCG. *J. Antimicrob. Chemother*. 46, 917-919 (2000)
21. Mayuri, Bagchi, G., Das, T. K. & J. S. Tyagi: Molecular analysis of the dormancy response in *Mycobacterium smegmatis:* expression analysis of genes encoding the DevR-DevS two-component system, Rv3134c and chaperone alpha-crystallin homologues. *FEMS Microbiol. Lett.* 211, 231-237 (2002)
22. Sun, Z. & Y. Zhang: Spent culture supernatant of Mycobacterium tuberculosis H37Ra improves viability of aged cultures of this strain and allows small inocula to initiate growth. *J. Bacteriol.* 181, 7626-7628 (1999)
23. Zhang, Y., Y. Yang, A. Woods, R. J. Cotter & Z. Sun: Resuscitation of dormant *Mycobacterium tuberculosis* by phospholipids or specific peptides. *Biochem. Biophys. Res. Commun.* 284, 542-547 (2001)
24. Mukamolova, G. V., O. A. Turapov, D. I. Young, A. S. Kaprelyants, D. B. Kell, & M. Young: A family of autocrine growth factors in *Mycobacterium tuberculosis*. *Mol. Microbiol.* 46, 623-635 (2002)
25. Biketov, S., G. V. Mukamolova, V. Potapov, E. Gilenkov, G. Vostroknutova, D. B. Kell, M. Young & A. S. Kaprelyants: Culturability of *Mycobacterium tuberculosis* cells isolated from murine macrophages: a bacterial growth factor promotes recovery. *FEMS Immunol. Med. Microbiol.* 29, 233-240 (2000)
26. McCune, R. M., R. Tompsett & W. McDermott: The fate of Mycobacterium tuberculosis in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculous infection to the latent state by administration of pyrazinamide and a companion drug. *J. Exp. Med.* 104, 763-802 (1956)
27. McCune, R., S. H. Lee, K. Deuschle & W. McDermott: Ineffectiveness of isoniazid in modifying the phenomenon of microbial persistence. *Am. Rev. Tuberc.* 76, 1106-1109 (1957)
28. Orme I. M.: The latent tubercle bacillus (I'll let you know if I ever meet one). *Int. J. Tuberc. Lung Dis.* 5, 589-593 (2001)
29. Mazurek, G. H., P. A. LoBue, C. L. Daley, J. Bernardo, A. A. Lardizabal, W. R. Bishai, M. F. Iademarco & J. S. Rothel: Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. *JAMA* 286, 1740-1747 (2001)
30. Mazurek, G. H. & M. E. Villarino: Guidelines for using the QuantiFERON-TB test for diagnosing latent *Mycobacterium tuberculosis* infection. Centers for Disease Control and Prevention. *MMWR Recomm. Rep*. 52(RR-2), 15-18 (2003)
31. Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am. J. Respir. Crit. Care Med*. 161, S221-247 (2000)
32. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. *MMWR Recomm. Rep.* 49(RR-6), 1-51 (2000)

33. Lincoln, E. M.: Effect of antimicrobial therapy on prognosis of primary tuberculosis in childhood. *Am. Rev. Tuberc.*69, 682-689 (1954)
34. Ferebee, S. H., F. W. Mount & A. A. Anastasiades: Prophylactic effects of isoniazid on primary tuberculosis in children. A preliminary report. *Am. Rev. Tuberc.* 76, 942-963 (1957)
35. Comstock, G. W., S. H. Ferebee & L. M. Hammes: A controlled trial of community-wide isoniazid prophylaxis in Alaska. *Am. Rev. Respir. Dis.* 95, 935-943 (1967)
36. Bate, J.: The tuberculin skin test and treatment of latent tuberculosis infection, in TUBERCULOSIS, 2nd ed, Ed., William N. Rom and Stuart Garay, Chapter 55, p. 837-842, Lippincott Williams & Wilkins, A Wolters Kluwer Company, New York (2003)
37. Hill, A. R.: "Treatment of LTBI": selling point or semantic blunder? *Am. J. Respir. Crit. Care Med.* 165, 1338 (2002)
38. Katz, J., S. Kunofsky, V. Damijonaitis, A. Lafleur & T. Caron: Effect of isoniazid upon the reactivation of inactive tuberculosis; final report. *Am. Rev. Respir. Dis*. 91, 345-350 (1965)
39. Zhang, Y.: Isoniazid, in TUBERCULOSIS, 2nd ed, Ed., William N. Rom and Stuart Garay, Chapter 49, pp739-758, Lippincott Williams & Wilkins, A Wolters Kluwer Company, New York (2003)
40. Moat, A. G. & J. W. Foster: Microbial Physiology, Third edition, Wiley-Liss, A John Wiley & Sons, Inc. Publication, New York (1995)
41. Kolter, R, D. A. Siegele & A. Tormo: The stationary phase of the bacterial life cycle. *Annu. Rev. Microbiol.* 47, 855-874 (1993)
42. Zambrano, M. M. & R. Kolter: GASPing for life in stationary phase. *Cell* 86, 181-184 (1996)
43. Smeulders, M. J., J. Keer, R. A. Speight & H. D. Williams: Adaptation of *Mycobacterium smegmatis* to stationary phase. *J. Bacteriol.* 181, 270-283 (1999)
44. Rychlik, I., L. Cardova, M. Sevcik & P. A. Barrow: Flow cytometry characterisation of *Salmonella typhimurium* mutants defective in proton translocating proteins and stationary-phase growth phenotype. *J. Microbiol. Methods* 42, 255-263 (2000)
45. Kaprelyants, A. S. & D. B. Kell: Dormancy in stationary-phase cultures of Micrococcus luteus: flow cytometric analysis of starvation and resuscitation. *Appl. Environ. Microbiol.* 59, 3187-3196 (1993)
46. Mukamolova, G. V., A. S. Kaprelyants, D. I. Young, M. Young & D. B. Kell: A bacterial cytokine. *Proc. Natl. Acad. Sci. USA* 95, 8916-8921 (1998)
47. Nystrom, T.: Nonculturable bacteria: programmed survival forms or cells at death's door? *Bioessays* 25, 204-211 (2003)
48. Roszak, D.B. & R. R. Colwell: Metabolic activity of bacterial cells enumerated by direct viable count. *Appl. Environ. Microbiol.* 53, 2889-2893 (1987)
49. Wilson, G. S.: The proportion of viable bacteria in young cultures with especial reference to the technique employed in counting. *J. Bacteriol.* 7, 405-445 (1922)
50. Shleeva, M. O., K. Bagramyan, M. V. Telkov, G. V. Mukamolova, M. Young, D. B. Kell & A. S. Kaprelyants: Formation and resuscitation of "non-culturable" cells of Rhodococcus rhodochrous and *Mycobacterium tuberculosis* in prolonged stationary phase. Microbiology 148, 1581-1591 (2002)
51. Canetti, G.: The Tubercle Bacillus in the Pulmonary Lesion of Man. Springer Publishing Company, New York (1955)
52. Hobby, G. L., O. Auerbach, T. F. Lenert, M. J. Small & J. V. Comer: The late emergence of *M. tuberculosis* in liquid cultures of pulmonary lesions resected from humans. *Am. Rev. Tuberc.* 70, 191-218 (1954).
53. Medlar, E. M., S. Berstein, & D. M. Steward: A bacteriologic study of resected tuberculous lesions. *Am. Rev. Tuberc.* 66, 36-43 (1952)
54. Beck, F. & D. Yegian: A study of the tubercle bacillus in resected pulmonary lesions. *Am. Rev. Tuberc.* 66, 44-51 (1952).
55. Falk, A., W. B. Tucker, & J. E. Kaufman: A clinical and bacteriologic correlation of resected pulmonary tuberculous lesions. *Am. Rev. Tuberc.* 70, 689-700 (1954)
56. Wayne, L. G.: The bacteriology of resected tuberculous pulmonary lesions. II. Observations on bacilli which are stainable but which cannot be cultured. Am. Rev. Respir. Dis. 82, 370-377 (1960).
57. Much, H.: Die nach Ziel nicht darstellbaren Formen des Tuberkelbacillus. *Berlin Klin. Wochenschr*. 45, 691-694 (1908).
58. Sweany, H. C.: The granules of the tubercle bacillus. *Am. Rev. Tuberc.* 17, 53-76 (1928)
59. Sweany, H. C.: The filterability of the tubercle bacillus. *Am. Rev. Tuberc.* 17, 77-85 (1928)
60. Khomenko, A. G.: The variability of *Mycobacterium tuberculosis* in patients with cavitary pulmonary tuberculosis in the course of chemotherapy. *Tubercle* 68, 243-253 (1987)
61. Ratnam, S. & S. Chandrasekhar: The pathogenicity of spheroplasts of *Mycobacterium tuberculosis*. *Am. Rev. Respir. Dis.* 114, 549-554 (1976)
62. Chandrasekhar, S. & S. Ratnam: Studies on cell-wall deficient non-acid fast variants of *Mycobacterium tuberculosis. Tuberc. Lung. Dis.* 73, 273-279 (1992)
63. Domingue, G. J. & H. B. Woody: Bacterial persistence and expression of disease. *Clin. Microbiol. Rev*. 10, 320-344 (1997)
64. Fontes, A.: Uber die Filtrierbarkeit des Tuberkelvirus vom Standpunkte des Polymorphismus. *Beitr. Klin. Tuberk.* 77, 1-15 (1931)
65. Mellon, R. R. & L. W. Fisher: New studies on the filterability of pure cultures of the tubercle group of microorganisms. *J. Infect. Dis.* 51, 117-128 (1932).
66. Hernandez-Pando, R., M. Jeyanathan, G. Mengistu, D. Aguilar, H. Orozco, M. Harboe, G. A. Rook & G. Bjune: Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. *Lancet* 356, 2133-2138 (2000).
67. de Wit, D., M. Wootton, J. Dhillon & D. A. Mitchison: The bacterial DNA content of mouse organs in the Cornell model of dormant tuberculosis. *Tuberc. Lung. Dis.* 76, 555-562 (1995)
68. Pai, S. R., J. K. Actor, E. Sepulveda, R. L. Hunter & C. Jagannath: Identification of viable and non-viable *Mycobacterium tuberculosis* in mouse organs by directed RT-PCR for antigen 85B mRNA. *Microb. Pathog*. 28, 335-342 (2000)
69. Daley, C. Tuberculosis latency in humans, in TUBERCULOSIS – 2nd ed, Ed. William N. Rom and

Stuart Garay, Chapter 7, p. 85-99, Lippincott Williams & Wilkins, A Wolters Kluwer Company, New York (2003)
70. McCune, R. M., R. Tompsett & W. McDermott: The fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculous infection to the latent state by administration of pyrazinamide and a companion drug. *J. Exp. Med.* 104, 763-802 (1956)
71. McCune, R. M., F. M. Feldman, H. Lambert & W. McDermott: Microbial persistence I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. *J. Exp. Med.* 123, 445-468 (1966)
72. Scanga, C.A., V. P. Mohan, H. Joseph, K. Yu, J. Chan & J. L. Flynn: Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect. Immun*. 67, 4531-4538 (1999)
73. Grosset, J., C. Truffot-Pernot, C. Lacroix & B. Ji: Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. *Antimicrob. Agents Chemother*. 36, 548-551 (1992)
74. Wayne, L. G.: Dynamics of submerged growth of *Mycobacterium tuberculosis* under aerobic and microaerophilic conditions. *Am. Rev. Respir. Dis*. 114, 807-811 (1976)
75. Wayne, L. G. & K. Y. Lin: Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions. *Infect. Immun*. 37,1042-1049 (1982)
76. Wayne, L. G. & L. G. Hayes: An *in vitro* model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect. Immun*. 64, 2062-2069 (1996)
77. Wayne, L. G. & L. G. Hayes: Nitrate reduction as a marker for hypoxic shiftdown of *Mycobacterium tuberculosis. Tuberc. Lung Dis.*79, 127-132 (1998)
78. Hu, Y. M., P. D. Butcher, K. Sole, D. A. Mitchison & A. R. Coates: Protein synthesis is shutdown in dormant *Mycobacterium tuberculosis* and is reversed by oxygen or heat shock. *FEMS Microbiol. Lett.* 158, 139-145 (1998)
79. Hu, Y., J. A. Mangan, J. Dhillon, K. M. Sole, D. A. Mitchison, P. D. Butcher & A. R. Coates: Detection of mRNA transcripts and active transcription in persistent *Mycobacterium tuberculosis* induced by exposure to rifampin or pyrazinamide. *J. Bacteriol.* 182, 6358-6365 (2000)
80. McKinney, J. D., K. Höner Zu Bentrup, E. J. Munoz-Elias, A. Miczak, B. Chen, W. T. Chan, D. Swenson, J. C. Sacchettini, W. R. Jacobs, Jr.& D. G. Russell: Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 406, 735-738 (2000)
81. Hong, P. C., R. M. Tsolis & T. A. Ficht: Identification of genes required for chronic persistence of *Brucella abortus* in mice. *Infect. Immun.* 68, 4102-4107 (2000)
82. Dhillon, J., B. W. Allen, Y. M. Hu, A. R. Coates & D. A. Mitchison: Metronidazole has no antibacterial effect in Cornell model murine tuberculosis. *Int. J. Tuberc. Lung Dis.* 2, 736-742 (1998)
83. Brooks, J. V., S. K. Furney & I. M. Orme: Metronidazole therapy in mice infected with tuberculosis. *Antimicrob. Agents Chemother.* 43, 1285-1288 (1999)
84. DeMaio, J., Y. Zhang, C. Ko, D. B. Young & W. R. Bishai: A stationary-phase stress-response sigma factor from *Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. U S A.* 93, 2790-2794 (1996)
85. DeMaio. J., Y. Zhang, C. Ko & W. R. Bishai: *Mycobacterium tuberculosis sigF* is part of a gene cluster with similarities to the *Bacillus subtilis sigF* and *sigB* operons. *Tuberc. Lung Dis.*78, 3-12 (1997)
86. Beaucher, J., S. Rodrigue, P. E. Jacques, I. Smith, R. Brzezinski & L. Gaudreau: Novel *Mycobacterium tuberculosis* anti-sigma factor antagonists control sigmaF activity by distinct mechanisms. *Mol. Microbiol.* 45, 1527-1540 (2002)
87. Michele, T. M., C. Ko & W. R. Bishai: Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis sigF* gene: implications for chemotherapy against mycobacterial persistors. *Antimicrob. Agents Chemother.* 43, 218-225 (1999)
88. Chen, P., R. E. Ruiz, Q. Li, R. F. Silver & W. R. Bishai: Construction and characterization of a *Mycobacterium tuberculosis* mutant lacking the alternate sigma factor gene, *sigF. Infect. Immun*. 68, 5575-5580 (2000)
89. Manganelli, R., E. Dubnau, S. Tyagi, F. R. Kramer & I. Smith: Differential expression of 10 sigma factor genes in *Mycobacterium tuberculosis. Mol. Microbiol*. 31, 715-724 (1999)
90. Hu, Y. & A. R. Coates: Increased levels of sigJ mRNA in late stationary phase cultures of *Mycobacterium tuberculosis* detected by DNA array hybridisation. *FEMS Microbiol. Lett.* 202, 59-65 (2001)
91. Sherman, D. R., M. Voskuil, D. Schnappinger, R. Liao, M. I. Harrell & G. K. Schoolnik Regulation of the *M. tuberculosis* hypoxic response gene alpha-crystallin. *Proc. Natl. Acad. Sci. USA* 98, 7534-7539 (2001)
92. Graham, J. E. & J. E. Clark-Curtiss: Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). *Proc. Natl. Acad. Sci. USA* 96, 11554-11559 (1999)
93. Höner Zu Bentrup, K., A. Miczak, D. L. Swenson & D. G. Russell. Characterization of activity and expression of isocitrate lyase in *Mycobacterium avium* and *Mycobacterium tuberculosis. J. Bacteriol.* 181, 7161-7167 (1999)
94. Fenhalls, G., L. Stevens, L. Moses, J. Bezuidenhout, J. C. Betts, P. van Helden, P. T. Lukey & K. Duncan: *In situ* detection of *Mycobacterium tuberculosis* transcripts in human lung granulomas reveals differential gene expression in necrotic lesions. *Infect. Immun*. 70, 6330-6338 (2002)
95. Lorenz, M. C. & G. R. Fink: The glyoxylate cycle is required for fungal virulence. *Nature* 412, 83-86 (2001)
96. Glickman, M. S., J. S. Cox & W. R. Jacobs Jr.: A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of *Mycobacterium tuberculosis. Mol. Cell* 5, 717-727 (2000)
97. Yuan, Y., R. E. Lee, G. S. Besra, J. T. Belisle & C. E. Barry3rd: Identification of a gene involved in the biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. U S A* 92,6630-6634 (1995)
98. Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C.

E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M. A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead, & B. G. Barrell. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537-544 (1998)

99. Young, R. A., B. R. Bloom, C. M. Grosskinsky, J. Ivanyi, D. Thomas & R. W. Davis: Dissection of *Mycobacterium tuberculosis* antigens using recombinant DNA. *Proc. Natl. Acad. Sci. U S A* 82, 2583-2587 (1985)

100. Verbon, A., R. A. Hartskeerl, A. Schuitema, A. H. Kolk, D. B. Young & R. Lathigra: The 14,000-molecular-weight antigen of *Mycobacterium tuberculosis* is related to the alpha-crystallin family of low-molecular-weight heat shock proteins. *J. Bacteriol.* 174, 1352-1359 (1992)

101. Yuan, Y., D. D. Crane, C. E. Barry3rd: Stationary phase-associated protein expression in *Mycobacterium tuberculosis:* function of the mycobacterial alpha-crystallin homolog. *J. Bacteriol.* 178, 4484-4492 (1996)

102. Cunningham, A. F. & C. L. Spreadbury: Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. *J. Bacteriol*. 180, 801-808 (1998)

103. Yuan, Y., D. D. Crane, R. M. Simpson, Y. Q. Zhu, M. J. Hickey, D. R. Sherman & C. E. Barry 3rd: The 16-kDa alpha-crystallin (Acr) protein of *Mycobacterium tuberculosis* is required for growth in macrophages. *Proc. Natl. Acad. Sci. U S A* 95, 9578-9583 (1998)

104. Desjardin, L. E.,L. G. Hayes, C. D. Sohaskey, L. G. Wayne & K. D. Eisenach: Microaerophilic induction of the alpha-crystallin chaperone protein homologue (*hspX*) mRNA of *Mycobacterium tuberculosis. J. Bacteriol.* 183, 5311-5316 (2001)

105. Garbe, T. R., N. S. Hibler & V. Deretic: Response to reactive nitrogen intermediates in *Mycobacterium tuberculosis:* induction of the 16-kilodalton alpha-crystallin homolog by exposure to nitric oxide donors. *Infect. Immun*. 67, 460-465 (1999)

106. Monahan, I. M., J. Betts, D. K. Banerjee & P. D. Butcher: Differential expression of mycobacterial proteins following phagocytosis by macrophages. *Microbiology* 147, 459-471 (2001)

107. Boon, C. & T. Dick: *Mycobacterium bovis* BCG response regulator essential for hypoxic dormancy. *J. Bacteriol*. 184, 6760-6767 (2002)

108. Cashel, M., D. R. Gentry, V. J. Hernandez & D. Vinella: The stringent response, p. 1488-1496. In F. C. Neidhardt, R. Curtis, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C. (1996)

109. Avarbock, D., J. Salem, L. S. Li, Z. M. Wang & H. Rubin: Cloning and characterization of a bifunctional RelA/SpoT homologue from *Mycobacterium tuberculosis. Gene* 233, 261-269 (1999)

110. Primm, T. P., S. J. Andersen, V. Mizrahi, D. Avarbock, H. Rubin H & C. E. Barry 3rd: The stringent response of *Mycobacterium tuberculosis* is required for long-term survival. *J. Bacteriol*. 182, 4889-4898 (2000)

111. Dahl, J. L., C. N. Kraus, H. I. Boshoff, B. Doan, K. Foley, D. Avarbock, G. Kaplan, V. Mizrahi, H. Rubin & C. E. Barry 3rd: The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of *Mycobacterium tuberculosis* in mice. *Proc. Natl. Acad. Sci. U S A* 100, 10026-10031 (2003)

112. Ramakrishnan, L., N. A. Federspiel & S. Falkow: Granuloma-specific expression of mycobacterium virulence proteins from the glycine-rich PE-PGRS family. *Science* 288, 1436-1439 (2000)

113. Espitia, C., J. P. Laclette, M. Mondragon-Palomino, A. Amador, J. Campuzano, A. Martens, M. Singh, R. Cicero, Y. Zhang & C. Moreno: The PE-PGRS glycine-rich proteins of Mycobacterium tuberculosis: a new family of fibronectin-binding proteins? *Microbiology* 145, 3487-3495 (1999)

114. Brennan, M.J., G. Delogu, Y. Chen, S. Bardarov, J. Kriakov, M. Alavi & W. R. Jacobs Jr: Evidence that mycobacterial PE-PGRS proteins are cell surface constituents that influence interactions with other cells. *Infect. Immun.* 69, 7326-7633 (2001)

115. Banu, S., N. Honore, B. Saint-Joanis, D. Philpott, M. C. Prevost & S. T. Cole. Are the PE-PGRS proteins of *Mycobacterium tuberculosis* variable surface antigens? *Mol. Microbiol.* 44, 9-19 (2002)

116. Brennan, M. J. & G. Delogu: The PE multigene family: a 'molecular mantra' for mycobacteria. *Trends Microbiol.* 10, 246-249 (2002)

117. Lim, A., M. Eleuterio, B. Hutter, B. Murugasu-Oei & T. Dick: Oxygen depletion-induced dormancy in *Mycobacterium bovis* BCG. *J. Bacteriol.* 181, 2252-2256 (1999)

118. Dasgupta, N., V. Kapur, K. K. Singh, T. K. Das, S. Sachdeva, K. Jyothisri & J. S. Tyagi: Characterization of a two-component system, *devR-devS*, of *Mycobacterium tuberculosis. Tuberc. Lung Dis*. 80, 141-159 (2000)

119. Park, H. D., K. M. Guinn, M. I. Harrell, R. Liao, M. I. Voskuil, M. Tompa, G. K. Schoolnik & D. R. Sherman: Rv3133c/dosR is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis. Mol. Microbiol.* 48, 833-843 (2003)

120. Parish, T., D. A. Smith, S. Kendall, N. Casali, G. J. Bancroft & N. G. Stoker: Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis. Infect. Immun*. 71, 1134-1140 (2003)

121. O'Toole, R., M. J. Smeulders, M. C. Blokpoel, E. J. Kay, K. Lougheed K & H. D. Williams: A two-component regulator of universal stress protein expression and adaptation to oxygen starvation in *Mycobacterium smegmatis. J. Bacteriol.* 185, 1543-1554 (2003)

122. Hutter, B. & T. Dick. Up-regulation of narX, encoding a putative 'fused nitrate reductase' in anaerobic dormant *Mycobacterium bovis* BCG. *FEMS Microbiol. Lett.* 178, 63-69 (1999)

123. Hutter, B. & T. Dick: Analysis of the dormancy-inducible *narK2* promoter in *Mycobacterium bovis* BCG. FEMS Microbiol. Lett. 188, 141-146 (2000)

124. Fritz, C., S. Maass, A. Kreft & F. C. Bange: Dependence of *Mycobacterium bovis* BCG on anaerobic nitrate reductase for persistence is tissue specific. *Infect. Immun*. 70, 286-291 (2002)
125. Rosenkrands, I., R. A. Slayden, J. Crawford, C. Aagaard, C. E. Barry 3rd & P. Andersen: Hypoxic response of *Mycobacterium tuberculosis* studied by metabolic labeling and proteome analysis of cellular and extracellular proteins. *J. Bacteriol*. 184, 3485-3491 (2002)
126. Betts, J. C., P. T. Lukey, L. C. Robb, R. A. McAdam & K. Duncan: Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. *Mol. Microbiol*. 43, 717-731 (2002)
127. Dubos, R. J., & B. D. Davis: Factors affecting the growth of tubercle bacilli in liquid media. *J. Exp. Med*. 83, 409-423 (1946)
128. Hobby, G. L., K. Meyer & E. Chaffee: Observations on the mechanism of penicillin. *Proc. Soc. Exper. Biol. Med.* 50, 281-285 (1942).
129. Kondo, E. & K. Kanai: [Studies on the relationship between the proliferation rate of infecting tubercle bacilli and the effectiveness of chemotherapy. I. Observations in a mouse experimental model using a streptomycin-dependent strain (author's transl)]. *Kekkaku* 52, 411-415 (1977)
130. Stewart, P. S.: Mechanisms of antibiotic resistance in bacterial biofilms. *Int. J. Med. Microbiol.* 292, 107-113 (2002)
131. Hogan, D. & R. Kolter: Why are bacteria refractory to antimicrobials? *Curr. Opin. Microbiol*. 5, 472-477 (2002)
132. Lewis, K.: Riddle of biofilm resistance. *Antimicrob. Agents Chemother*. 45, 999-1007 (2001)
133. Zhang, Y. & L. M. Amzel: Tuberculosis drug targets. *Curr. Drug Targets* 3, 131-154 (2002)
134. Greenway, D. L. & R. R. England: The intrinsic resistance of *Escherichia coli* to various antimicrobial agents requires ppGpp and sigma S. *Lett. Appl. Microbiol*. 29, 323-326 (1999)
135. Demerec, M.: Origin of bacterial resistance to antibiotics. *J. Bacteriol.* 56, 63-74 (1948)
136. Booth, I. R.: Stress and the single cell: intrapopulation diversity is a mechanism to ensure survival upon exposure to stress. *Int. J. Food Microbiol.* 78, 19-30 (2002)
137. Debbia, E. A., S. Roveta, A. M. Schito, L. Gualco & A. Marchese: Antibiotic persistence: the role of spontaneous DNA repair response. *Microb. Drug Resist.* 7, 335-342 (2001)
138. Mitchison, D. A.: Treatment of tuberculosis. The Mitchell lecture 1979. *J. R. Coll. Physicians Lond.* 14, 91-95, 98-99 (1980)
139. Jindani, A., V. R. Aber, E. A. Edwards & D. A. Mitchison: The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am. Rev. Respir. Dis.* 121, 939-949 (1980)
140. Wallis, R. S., S. Patil, S. H. Cheon, K. Edmonds, M. Phillips, M. D. Perkins, M. Joloba, A. Namale, J. L. Johnson, L. Teixeira, R. Dietze, S. Siddiqi, R. D. Mugerwa, K. Eisenach & J. J. Ellner: Drug tolerance in *Mycobacterium tuberculosis. Antimicrob. Agents Chemother*. 43, 2600-2606 (1999)
141. Pagel, W.: Reactivation of a tuberculous focus by microorganisms other than the tubercle bacillus. *Lancet* 1, 1279-1280 (1937)
142. Controlled clinical trial of five short-course (4-month) chemotherapy regimens in pulmonary tuberculosis. First report of 4th study. East African and British Medical Research Councils. *Lancet* 2(8085), 334-338 (1978)
143. A controlled clinical trial of 3- and 5-month regimens in the treatment of sputum-positive pulmonary tuberculosis in South India. Tuberculosis Research Centre, Madras, and National Tuberculosis Institute, Bangalore. *Am. Rev. Respir. Dis.* 134, 27-33 (1986)
144. Balasubramanian, R., S. Sivasubramanian, V. K. Vijayan, R. Ramachandran, M. S. Jawahar, C. N. Paramasivan, N. Selvakumar & P. R. Somasundaram: Five year results of a 3-month and two 5-month regimens for the treatment of sputum-positive pulmonary tuberculosis in south India. *Tubercle* 71, 253-258 (1990)
145. Orme, I. M.: A mouse model of the recrudescence of latent tuberculosis in the elderly. *Am. Rev. Respir. Dis*. 137, 716-718 (1988)
146. Adams, L. B., C. M. Mason, J. K. Kolls, D. Scollard, J. L. Krahenbuhl & S. Nelson: Exacerbation of acute and chronic murine tuberculosis by administration of a tumor necrosis factor receptor-expressing adenovirus. *J. Infect. Dis.* 171, 400-405 (1995)
147. Arriaga, A. K., E. H. Orozco, L. D. Aguilar, G. A. Rook & R. Hernandez Pando: Immunological and pathological comparative analysis between experimental latent tuberculous infection and progressive pulmonary tuberculosis. *Clin. Exp. Immunol*. 128, 229-237 (2002)
148. Brown, D. H., B. A. Miles & B. S. Zwilling: Growth of Mycobacterium tuberculosis in BCG-resistant and -susceptible mice: establishment of latency and reactivation. *Infect. Immun.* 63, 2243-2247 (1995)
149. Capuano, S. V., D. A. Croix, S. Pawar, A. Zinovik, A. Myers, P. L. Lin, S. Bissel, C. Fuhrman, E. Klein & J. L. Flynn: Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. *Infect. Immun.* 71, 5831-5844 (2003)
150. Lillebaek, T., A. Dirksen, I. Baess, B. Strunge, V. O. Thomsen & A. B. Andersen: Molecular evidence of endogenous reactivation of *Mycobacterium tuberculosis* after 33 years of latent infection. *J. Infect. Dis*. 185, 401-404 (2002)
151. Cox, J. H., B. C. Knight & J. Ivanyi: Mechanisms of recrudescence of *Mycobacterium bovis* BCG infection in mice. *Infect. Immun.* 57, 1719-1724 (1989)
152. Scanga, C.A., V. P. Mohan, K. Yu, H. Joseph, K. Tanaka, J. Chan & J. L. Flynn: Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon gamma and nitric oxide synthase 2. *J. Exp. Med.* 192, 347-358 (2000)
153. van Pinxteren, L. A., J. P. Cassidy, B. H. Smedegaard, E. M. Agger & P. Andersen: Control of latent *Mycobacterium tuberculosis* infection is dependent on CD8 T cells. *Eur. J. Immunol.* 30, 3689-3698 (2000)
154. Howard, A. D. & B. S. Zwilling: Reactivation of tuberculosis is associated with a shift from type 1 to type 2 cytokines. *Clin. Exp. Immunol.* 115, 428-434 (1999)
155. Bodnar, K. A., N. V. Serbina & J. L. Flynn: Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect. Immun.* 69, 800-809 (2001)
156. Pancholi, P., A. Mirza, N. Bhardwaj & R. M. Steinman: Sequestration from immune CD4+ T cells of

mycobacteria growing in human macrophages. *Science* 260, 984-986. (1993)
157. Hmama, Z., R. Gabathuler, W. A. Jefferies, G. de Jong & N. E. Reiner: Attenuation of HLA-DR expression by mononuclear phagocytes infected with *Mycobacterium tuberculosis* is related to intracellular sequestration of immature class II heterodimers. *J. Immunol.* 161, 4882-4893 (1998)
158. Noss, E. H., C. V. Harding & W. H. Boom:. *Mycobacterium tuberculosis* inhibits MHC class II antigen processing in murine bone marrow macrophages. *Cell. Immunol.* 201, 63-74 (2000)
159. Hirsch, C. S., R. Hussain, Z. Toossi, G. Dawood, F. Shahid & J. J. Ellner. Cross-modulation by transforming growth factor beta in human tuberculosis: suppression of antigen-driven blastogenesis and interferon gamma production. *Proc. Natl. Acad. Sci. USA* 93, 3193-3198 (1996)
160. Rojas, R. E., K. N. Balaji, A. Subramanian & W. H. Boom: Regulation of human CD4(+) alphabeta T-cell-receptor-positive (TCR(+)) and gammadelta TCR(+) T-cell responses to *Mycobacterium tuberculosis* by interleukin-10 and transforming growth factor beta. *Infect. Immun*. 67, 6461-6472 (1999)
161. Turner, J., M. Gonzalez-Juarrero, D. L. Ellis, R. J. Basaraba, A. Kipnis, I. M. Orme & A. M. Cooper: *In vivo* IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J. Immunol.* 169, 6343-6351 (2002)
162. Hernandez-Garay, M. & P. Mendez-Samperio: Transforming growth factor-beta decreases survival of *Mycobacterium bovis*-activated T cells. Arch. Med. Res. 34, 20-25 (2003)
163. Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach & D. L. Sacks: CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* 420, 502-507 (2002)
164. Hernandez-Pando, R. & G. A. Rook: The role of TNF-alpha in T-cell-mediated inflammation depends on the Th1/Th2 cytokine balance. *Immunology* 82, 591-595 (1994)
165. Flynn, J. L., M. M. Goldstein, J. Chan, K. J. Triebold, K. Pfeffer, C. J. Lowenstein, R. Schreiber, T. W. Mak & B. R. Bloom: Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 2, 561-572 (1995)
166. Bean, A. G., D. R. Roach, H. Briscoe, M. P. France, H. Korner, J. D. Sedgwick & W. J. Britton: Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin. *J. Immunol.* 162, 3504-3511 (1999)
167. Mohan, V.P., C. A. Scanga, K. Yu, H. M. Scott, K. E. Tanaka, E. Tsang, M. M. Tsai, J. L. Flynn & J. Chan: Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. *Infect. Immun.* 69, 1847-1855 (2001)
168. Maini, R., E. W. St Clair, F. Breedveld, D. Furst, J. Kalden, M. Weisman, J. Smolen, P. Emery, G. Harriman, M. Feldmann & P. Lipsky: Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Study Group . *Lancet* 354(9194), 1932-1939 (1999)
169. Keane, J., S. Gershon, R. P. Wise, E. Mirabile-Levens, J. Kasznica, W. D. Schwieterman, J. N. Siegel & M. M. Braun: Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med*. 345,1098-1104 (2001)
170. Nunez Martinez, O., C. Ripoll Noiseux, J. A. Carneros Martin, V. Gonzalez Lara, H. G. Gregorio Maranon: Reactivation tuberculosis in a patient with anti-TNF-alpha treatment. *Am. J. Gastroenterol.* 96, 1665-1666 (2001)
171. Wagner, T. E., E. S. Huseby & J. S. Huseby: Exacerbation of *Mycobacterium tuberculosis* enteritis masquerading as Crohn's disease after treatment with a tumor necrosis factor-alpha inhibitor. *Am. J. Med*. 112, 67-69 (2002)
172. Mayordomo, L., J. L. Marenco, J. Gomez-Mateos & E. Rejon: Pulmonary miliary tuberculosis in a patient with anti-TNF-alpha treatment. *Scand. J. Rheumatol*. 31, 44-45 (2002)
173. Roth, S., E. Delmont, P. Heudier, R. Kaphan, E. Cua, J. Castela, J. M. Verdier, R. M. Chichmanian & J. G. Fuzibet: Anti-TNF alpha monoclonal antibodies (infliximab) and tuberculosis: apropos of 3 cases [Article in French]. *Rev. Med. Interne*. 23, 312-316 (2002)
174. Chan, J., Y. Xing, R. S. Magliozzo & B. R. Bloom: Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med*. 175, 1111-1122 (1992)
175. Flynn, J. L., C. A. Scanga, K. E. Tanaka & J. Chan: Effects of aminoguanidine on latent murine tuberculosis. *J. Immunol.* 160, 1796-1803 (1998)
176. Botha, T. & B. Ryffel: Reactivation of latent tuberculosis by an inhibitor of inducible nitric oxide synthase in an aerosol murine model. *Immunology* 107, 350-357 (2002)
177. Berle, B. B.: Emotional factors and tuberculosis: A critical review of the literature. *Psychosom. Med.*10, 366-373 (1948)
178. Wittkower, E. D.: Psychological aspects of tuberculosis. *Am. Rev. Tuberc.* 67, 869-873 (1953)
179. Clarke, E. R., D. W. Zahn & T. H. Holmes: The relationship of stress, adrenocortical function, and tuberculosis. *Am. Rev. Tuberc.* 69, 351-369 (1954)
180. Dubos, R. & J. Dubos: *The White Plague: Tuberculosis, Man, and Society.* Rutgers University Press, New Brunswick, N. J. (1987)
181. Ryan, F.: *The Forgotten Plague. How the Battle against Tuberculosis Was Won - and Lost,* p155-156, Little, Brown and Company (1993)
182. Pecyna, M. B. & A. Hryniewiecka: [The role of psychological factors in the etiopathogenesis of pulmonary tuberculosis] [Article in Polish] *Pol. Tyg. Lek.* 43, 850-853 (1988)
183. Rogowski, J., K. Mulak, W. Nartowska, Z. Piasecki & B. Kadrys: [Psychological and social factors in the recurrence of pulmonary tuberculosis][Article in Polish] *Pneumonol. Pol.* 48, 685-692 (1980)
184. Lukashova, E. N., L. N. Igisheva & I. F. Kopylova: [Psychological peculiarities in adolescent patients with tuberculosis][Article in Russian] *Probl. Tuberk.* 1, 39-41 (2002)

185. Dai, G., S. Phalen & D. N. McMurray: Nutritional modulation of host responses to mycobacteria. *Front. Biosci.* 3, E110-122 (1998)
186. Yang, E.V. & R. Glaser: Stress-induced immunomodulation and the implications for health. *Int. Immunopharmacol.* 2, 315-324 (2002)
187. Biondi, M. & L. G. Zannino: Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: a review. *Psychother. Psychosom.* 66, 3-26 (1997)
188. Wyser, C., G. Walzl, J. P. Smedema, F. Swart, E. M. van Schalkwyk & B. W. van de Wal: Corticosteroids in the treatment of tuberculous pleurisy. A double-blind, placebo-controlled, randomized study. *Chest* 110, 333-338 (1996)
189. Smego, R. A. & N. Ahmed: A systematic review of the adjunctive use of systemic corticosteroids for pulmonary tuberculosis. *Int. J. Tuberc. Lung Dis*. 7, 208-213 (2003)
190. Dooley, D. P., J. L. Carpenter & S. Rademacher: Adjunctive corticosteroid therapy for tuberculosis: a critical reappraisal of the literature. *Clin. Infect. Dis.* 25, 872-887 (1997)
191. Tam, L. S., E. K. Li, S. M. Wong & C. C. Szeto: Risk factors and clinical features for tuberculosis among patients with systemic lupus erythematosus in Hong Kong. *Scand. J. Rheumatol.* 31, 296-300 (2002)
192. Chan, Y. C. & G. Yosipovitch: Suggested guidelines for screening and management of tuberculosis in patients taking oral glucocorticoids--an important but often neglected issue. *J. Am. Acad. Dermatol*. 49, 91-95 (2003)
193. Hernandez-Pando, R., M. De La Luz Streber, H. Orozco, K. Arriaga, L. Pavon, S. A. Al-Nakhli & G. A. Rook: The effects of androstenediol and dehydroepiandrosterone on the course and cytokine profile of tuberculosis in BALB/c mice. *Immunology* 95, 234-241 (1998)
194. Yamagishi, F., Y. Sasaki, T. Yagi, H. Yamatani, F. Kuroda & H. Shoda: Frequency of complication of diabetes mellitus in pulmonary tuberculosis. *Kekkaku* 75, 435-437 (2000)
195. Kado, S., T. Isobe, S. Ishioka, M. Yamakido, Y. Shibata & T. Kuraoka: Clinical feature of the diabetic patients with recurrent pulmonary tuberculosis. *Kekkaku* 67, 313-318 (1992)
196. Wada, M.: The effectiveness of pyrazinamide-containing six-month short course chemotherapy. *Kekkaku* 75, 665-673 (2000)
197. Steger, R. W.& M. B. Rabe: The effect of diabetes mellitus on endocrine and reproductive function. *Proc. Soc. Exp. Biol. Med*. 214, 1-11 (1997)
198. Smurova, T. F.& I. L. Egorova: Endocrine disorders and principles of their correction in patients with pulmonary tuberculosis and concomitant diabetes mellitus. *Klin. Med. (Mosk)* 71, 58-62 (1993)
199. Chan, O., K. Inouye, M. C. Riddell, M. Vranic & S. G. Matthews: Diabetes and the hypothalamo-pituitary-adrenal (HPA) axis. *Minerva Endocrinol.* 28, 87-102 (2003)
200. Tsukaguchi, K., T. Yoneda, M. Yoshikawa, T. Tokuyama, A. Fu, K. Tomoda, N. Narita, Y. Enoki, M. Tsukaguchi & F. Shirai F *et al.*: Case study of interleukin-1 beta, tumor necrosis factor alpha and interleukin-6 production peripheral blood monocytes in patients with diabetes mellitus complicated by pulmonary tuberculosis. *Kekkaku* 67, 755-760 (1992)
201. Hernandez-Pando, R., L. Pavon, E. H. Orozco, J. Rangel & G. A. Rook: Interactions between hormone-mediated and vaccine-mediated immunotherapy for pulmonary tuberculosis in BALB/c mice. *Immunology* 100, 391-398 (2000)
202. Morgan, W.K.: The relationship between tuberculosis and silicosis. *Am. Rev. Respir. Dis*. 119, 319-320 (1979)
203. Vorwald, A. J., M. Dworski & P. C. Pratt: The use of quartz dust for challenging the viability of tubercle bacilli in tuberculous lesions. *Am. Rev. Respir. Dis*. 69, 841-842 (1954)
204. Allison, A. C. & P. D. Hart: Potentiation by silica of the growth of Mycobacterium tuberculosis in macrophage cultures. *Br. J. Exp. Pathol*. 49, 465-476 (1968)
205. Yucesoy, B., V. Vallyathan, D. P. Landsittel, D. S. Sharp, A. Weston, G. R. Burleson, P. Simeonova, M. McKinstry & M. I. Luster: Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis. *Toxicol. Appl. Pharmacol*. 172, 75-82 (2001)
206. Zhang, Y. & D. Mitchison: The curious characteristics of pyrazinamide: a review. *Int. J. Tuberc. Lung Dis*. 7, 6-21 (2003)
207. Zhang, Y., A. Scorpio, H. Nikaido & Z. Sun: Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. *J. Bacteriol*. 181, 2044-2049 (1999)
208. Zhang, Y., H. Zhang & Z. Sun: Susceptibility of *Mycobacterium tuberculosis* to weak acids. *J. Antimicrob. Chemother.* 52, 56-60 (2003)
209. Zhang, Y., M. W. Wade, A. Scorpio, H. Zhang & Z. Sun: Mode of action of pyrazinamide: Disruption of membrane potential and transport by pyrazinamide. *J. Antimicrob. Chemother.* 52, 790-795 (2003)
210. Sharma, V., S. Sharma, K. Hoener zu Bentrup, J. D. McKinney, D. G. Russell, W. R. Jacobs Jr & J. C. Sacchettini: Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis. Nat. Struct. Biol*. 7, 663-668 (2000)
211. Huang, C. C., C. V. Smith, M. S. Glickman, W. R. Jacobs Jr & J. C. Sacchettini: Crystal structures of mycolic acid cyclopropane synthases from *Mycobacterium tuberculosis. J. Biol. Chem.* 277, 11559-11569 (2002)
212. Brown, M. R. & A. W. Smith: Dormancy and persistence in chronic infection: role of the general stress response in resistance to chemotherapy. *J. Antimicrob. Chemother.* 48, 141-142 (2001)
213. Coates, A., Y. Hu, R. Bax and C. Page: The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discov*. 1, 895-910 (2002)
214. Lowrie, D. B., R. E. Tascon, V. L. Bonato, V. M. Lima, L. H. Faccioli, E. Stavropoulos, M. J. Colston, R. G. Hewinson, K. Moelling K & C. L. Silva: Therapy of tuberculosis in mice by DNA vaccination. *Nature* 400, 269-271 (1999)
215. Repique, C. J., A. Li, F. M. Collins & S. L. Morris: DNA immunization in a mouse model of latent tuberculosis: effect of DNA vaccination on reactivation of disease and on reinfection with a secondary challenge. *Infect. Immun.* 70, 3318-3323 (2002)

216. Taylor, J. L., O. C. Turner, R. J. Basaraba, J. T. Belisle, K. Huygen & I. M. Orme: Pulmonary necrosis resulting from DNA vaccination against tuberculosis. *Infect. Immun.* 71, 2192-2198 (2003)
217. Study of chemotherapy regimens of 5 and 7 months' duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in South India. *Tubercle* 64, 73-91 (1983)
218. Rook, G. A. & R. Hernandez-Pando: Immunological and endocrinological characteristics of tuberculosis that provide opportunities for immunotherapeutic intervention. *Novartis Found. Symp.* 217, 73-87 (1998)
219. Dhillon, J. & D. A. Mitchison: Effect of vaccines in a murine model of dormant tuberculosis. *Tuber. Lung Dis*. 75, 61-64 (1994)
220. Wu, X. *et al.*, Poster presented at the Fourth World Congress on Tuberculosis, June 3-5, Washington, D.C. (2002)
221. Holland, S. M.: Cytokine therapy of mycobacterial infections. *Adv. Intern. Med.* 45, 431-452 (2000)

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