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New insights into TB physiology suggest untapped therapeutic opportunities

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Summary

The current drug regimens used to treat tuberculosis are largely comprised of serendipitously discovered drugs that are combined based on clinical experience. Despite curing millions, these drug regimens are limited by the long course of therapy, the emergence of resistance, and permanent tissue damage. The last two decades have produced only a single new drug but have represented a renaissance in our understanding of the physiology of TB infection. The advent of mycobacterial genetics, sophisticated immunological methods, and imaging technologies has transformed our understanding of bacterial physiology as well as the contribution of the host response to disease outcome. Specific alterations in bacterial metabolism, heterogeneity in bacterial state, and drug penetration all limit the effectiveness of antimicrobial therapy. This review summarizes these new biological insights and discusses strategies to exploit them for the rational development of more effective therapeutics. Three general strategies are discussed. First, our emerging insight into bacterial physiology suggests new pathways that might be targeted to accelerate therapy. Second, we explore whether the concept of genetic synergy can be used to design effective combination therapies. Finally, we outline possible approaches to modulate the host response to accentuate antibiotic efficacy. These biology-driven strategies promise to produce more effective therapies.

Keywords

tuberculosis; drug discovery; antibiotics; host response; immunity

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The state of TB treatment

Of all bacterial diseases, tuberculosis (TB) continues to exert the highest toll on human health. Despite effective chemotherapeutic regimens that were developed decades ago, TB continues to afflict 8.6 million and kill 1.3 million every year (1). This disease remains a persistent public health threat for a variety of complex biological and sociological reasons. Primary among these reasons is the long, complex, and variably effective antibiotic regimen that is needed to treat the disease.

The current treatment regime for drug-sensitive tuberculosis was established in 1994 by the WHO and utilizes a cocktail of four antibiotics, isoniazid (INH) , rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB), that were discovered 50-60 years ago (2). Drugs are administered for at least six months and, to ensure compliance, each dose should be monitored by a healthcare worker. The delivery of this directly observed therapy (DOT) is both expensive and difficult to accomplish in the resource-limited settings where TB is most prevalent. Even if this regimen is faithfully delivered, relapse rates are estimated to be as high as 2-20% depending on risk factors, such as diabetes, human immunodeficiency virus (HIV) co-infection, and antibiotic resistance (3).

While it is true that only a single new drug for TB has been introduced over the past 40 years, the shortcomings of TB chemotherapy are not merely the result of a lack of scientific effort. Eradication of *Mycobacterium tuberculosis* (Mtb) is considerably more difficult than many other infections for a number of purely biological reasons including limited drug penetration into both host tissue and the bacteria, heterogeneity in bacterial metabolic states that alter antibiotic susceptibility (4-6), and the propensity of mycobacteria to enter into a quiescent state that generally limits drug efficacy (7-9). The lack of a single standard preclinical model that mimics human disease states has made these issues even more difficult to overcome.

Understanding the fundamental biology that underlies drug efficacy *in vivo* could foster rational strategies for improving TB treatment by designing drugs that inhibit pathways critical during infection. Unfortunately, our current drugs were developed without this insight, and we are still discovering the mechanisms that determine their activity. A classic example of this knowledge gap is PZA. Regimens containing RIF and PZA are the most effective in eradicating Mtb and preventing relapse and remain the foundation of TB therapy. PZA was discovered in 1952 as a result of parallel programs to optimize the anti-mycobacterial properties of nicotinamde, programs that also led to the development of INH and ethionamide (ETA) (10). PZA shows little activity in vitro except under specific acidic conditions, but is a potent bactericidal agent *in vivo* that synergizes with RIF. The reasons for PZA's remarkable potency *in vivo* and synergy with RIF are still being unraveled. It is possible that the compound's mechanism of action is responsible (11). PZA may act by a variety of mechanisms, including inhibition of the Mtb fatty acid synthase, the inhibition of trans-translation, or the neutralization of the membrane potential (12-15). PZA attains high concentrations in necrotic regions of the TB lesion which might also contribute to its *in vivo* activity (4).

The example of PZA highlights the serendipity that led to our current TB treatment regimen. A similar compound with weak *in vitro* activity and an unknown mechanism of action would be unlikely to progress in a modern drug development program. Nevertheless, when administered to a patient, this compound possesses potent sterilizing activity. Over the past two decades, our understanding of the physiology of both host and pathogen during TB disease and treatment has increased exponentially. This review will explore our current knowledge of Mtb physiology and antibiotic activity during infection and discuss new strategies to capitalize on this insight to more rationally design new drugs or drug combinations that that improve treatment.

Mtb physiology *in vivo*

Our understanding of TB disease progression is derived from careful histopathological studies of tissues from human surgical resection and autopsy specimens in conjunction with animal models (16). Initial Mtb infection occurs upon the inhalation of bacilli, which are engulfed by the major phagocytic cell of the airway, the alveolar macrophage, where replication initiates. This cell is likely to be a transient niche for Mtb. Within a matter of days, Mtb is found within recruited myeloid cells, both macrophages and dendritic cells in the mouse (17). During this initial phase of infection, the bacterium grows relatively rapidly inside of an increasing number of recruited cells. In both mice and nonhuman primate models, each infecting bacterium establishes an independent focus of infection (18). Even at this very early stage, the necrosis (also called ‘caseation’) characteristic of mature TB lesions can be found, presumably as a result of the death of infected cells.

Expansion of the bacterial population is restricted with the onset of adaptive immunity (18,19). A robust $CD4^+$ T-cell response is capable of arresting bacterial replication through the enhancement of the antimicrobial capacity of the parasitized macrophage (20,21). $CD8^+$ T-cell-mediated cytotoxicity may also play a role in restricting the intracellular niche of the bacterium (22). At sites of strong T-cell immunity, the majority of bacilli are thought to arrest their growth in a nonreplicating state, in which nominal metabolism continues but the bacterium is relatively insensitive to antibiotic treatment (23,24).

This relatively simple situation is complicated by the existence of distinct host microenvironments that influence both bacterial physiology and drug penetration (*Fig. 1*). As described above, this environmental heterogeneity varies temporally as the infection progresses. However, it is also clear that the bacterial environment varies spatially throughout the infected tissue. Chronic TB lesions are dynamic structures comprised of a variety of lymphocyte classes, differentially activated macrophages and dendritic cells, and neutrophils (25). These cells are often located between a caseous core of necrotic material and a fibrous cuff that contains the lesion. The exclusion of vasculature from necrotic areas creates regions of differing hypoxia (26). Bacteria can be found throughout these complex structures. Intracellular bacteria are found within all classes of myeloid cells in the cellular region. In addition, extracellular bacteria are found in the necrotic region, varying from a negligible number in small contained lesions, to a huge number in cavitary lesions that are responsible for most transmission (27). Mature TB lesions are often termed ‘granuloma’ due to the predominance of epitheliod macrophages (28). However, Mtb infection induces a very

characteristic manifestation of the granuloma, making the application of this term somewhat imprecise. Thus, we term these ‘TB lesions’.

The distinct microenvironments in which the bacteria are found might be expected to present different environments and impose distinct physiological states on the bacterium. This supposition is supported by the observation of phenotypic heterogeneity among bacteria recovered from human sputum, which consist of both cell-associated and free bacteria that are differentially antibiotic susceptible (29,30). Understanding the relationship between histopathological niche, bacterial physiology, and drug susceptibility is fundamental to the design of more effective therapies. Unfortunately, the human observational studies that produced this detailed description of TB disease are not capable of probing bacterial physiology at a resolution that is sufficient to inform drug development. For this reason, a variety of experimentally tractable animal models have been used to model the environments that Mtb encounters in the human. Before describing our current understanding of Mtb physiology during infection, we will discuss the strengths and limitations of current model systems. The major models that have been used for this purpose are described below, in order of increasing complexity.

Cell culture models are simplest systems used to mimic infection and are amenable to a variety of high throughput chemical and genetic screening methodologies (31-33), as well as detailed metabolic flux estimations (34). By definition, these systems only recapitulate the environment encountered by intracellular bacteria. In addition, it is likely that no single cell type in monoculture will accurately recapitulate the multicellular environment of a TB lesion. Indeed, whole-genome screens identified overlapping, but remarkably distinct Mtb mutants that display growth defects mouse macrophages versus intact mouse spleen (35).

A variety of whole animal models have been investigated as TB models, including frog, zebrafish, mouse, guinea pig, rabbit, and nonhuman primate. Each of these has been reviewed elsewhere (36-39). The mouse remains the most commonly used model, both because of its low cost and genetic tractability. While many of the hallmarks of TB are recapitulated in the mouse, TB lesions in standard laboratory strains, such as C57BL/6, fail to develop central necrosis and the majority of bacteria remain intracellular (37). More recently, a number of new mouse models have been identified that increase the diversity of disease states that can be produced in this species. One strategy has been to take advantage of the natural diversity that exists in the mouse. For example, a specific substrain of *M. domesticus* , C3HeB/FeJ, was found to be hypersusceptible to Mtb infection due to a mutation in the IPR1 gene that enhances macrophage necrosis (40). This mouse strain develops encapsulated necrotic lesions that are hypoxic and more closely resemble human cavitary disease (41). A second strategy involves a more systematic manipulation of the mouse and infection to produce altered histopathology. A subcutaneous Mtb infection can produce lung lesions that bear striking resemblance to those seen in primates, if a mouse prone to inflammation is used (42). This observation suggests that the histopathology of lesions is not an absolute characteristic of a species but, instead, reflects the relative timing of bacterial colonization of the lung and the priming of the adaptive immune response. In both of these experimental models, significant heterogeneity in lesions exists in individual animals, similar to the case in human.

There is clearly no shortage of models to investigate Mtb infection. The major challenge is relating the specific stresses defined in simple systems to the actual microenvironments encountered in the intact lung, and understanding how the bacterial adaptations to these stresses affect antibiotic activity. Our current understanding of these events is summarized below.

Growth rate

The most obvious physiological adaptation to different host compartments is altered replication rate. The average doubling time of Mtb in chronically infected mice has been estimated by two different methods to be greater than 96 hours (24,43). Modeling in TB growth and death dynamics in the nonhuman primate model is consistent with very limited bacterial replication in most TB lesions (18). As many antibiotics, particularly cell wall inhibitors, preferentially act on growing cells, the limited growth that occurs during infection is thought to be one of the major factors limiting TB treatment efficacy (44).

While growth rate is often considered as simply the sum of the various individual metabolic adaptations described below, this view is predicated on the assumption that cellular metabolism always maximizes growth rate. This may be a reasonable assumption in many bacteria that rely on rapid growth to compete with neighboring microbes for limited resources. However, in slowly growing organisms that are not forced to compete, such as Mtb, this assumption might not be applicable (7). Indeed, growth rate appears to be actively controlled in Mtb in response to stress. A variety of stresses, such as low pH, low oxygen, or iron limitation, triggers the sensor kinase DosRST and induces triglyceride accumulation via the induction of the tgs1 gene. This response effectively depletes acetyl CoA and restricts bacterial growth, and mutants lacking this system continue to grow and divide under conditions that limit the replication of wild type bacteria (45). These observations indicate that growth arrest is an active response to stress that is separable from the metabolic capacity to grow. Other growth-regulatory pathways, such as the stringent response, produce a similar drug tolerant state in other bacteria (46), but it remains unclear if the mycobacterial stringent response (47,48) produces the same effect.

Consistent with the concept that low growth rate reduces treatment efficacy, antibiotics that retain their activity against nonreplicating bacteria are components of the most efficacious regimens in animal models. These compounds include RIF, bedaquiline (BDQ), fluoroquinolones, and PZA (7,49). Based on these data, identification of new compounds that share or maximize this activity remains a major goal of many drug discovery efforts.

Respiration

Despite possessing genes that are usually dedicated to microaerophillic and anaerobic metabolism, Mtb does not grow without oxygen in any laboratory medium yet investigated. This observation, in conjunction with the essentiality of the TCA cycle and terminal cytochrome oxidase complexes, has led to the classification of Mtb as an obligate aerobe, and it is clear that respiration is a critical feature of Mtb physiology that is altered in different host microenvironments.

Oxygen is a preferred electron acceptor for Mtb. The importance and overlapping roles of the major aa3-type cytochrome c oxidase and high affinity bd oxidase during murine infections highlights the critical role of aerobic respiration during infection (50). However, both direct measurements of dissolved oxygen (51) and histochemical studies (26) indicate that oxygen is limiting in many TB lesions. Oxygen availability is clearly heterogeneous. Vascularization is apparent in the cellular region of a lesion, but the central necrotic core that harbors extracellular bacteria appears to be largely hypoxic. The host response to infection can also change respiratory capacity in less obvious but equally important ways. For example, nitric oxide (NO) production by macrophages can poison the respiratory chain and produce a transcriptional response in Mtb that is indistinguishable from hypoxia (52). At the same time, NO is reduced to nitrite and nitrate in the tissue, and the Mtb nitrate reductase genes are induced during infection (53). Nitrogen-based respiration appears insufficient to support replication, at least under defined *in vitro* conditions. However, exogenously supplied nitrate increases Mtb survival *in vitro* during mild acid stress (pH 5.5) or hypoxia, indicating that this function may be important for maintaining cellular redox, an important determinant of antibiotic activity (45,54-56).

In the gut, inflammation has been found to alter bacterial respiration in even more unexpected ways. Reactive oxygen species generated by the immune response produce long-lived S- and N-oxides that can serve as electron acceptors for molybdepterin-dependent TMAO oxidase complexes and facilitate anaerobic respiration (57). As a result, inflammation actually promotes the selective growth of gamma-proteobacteria that can perform these reactions (58). While similar observations have not been reported in the lung, it is notable that a homologous TMAO oxidase and molybdepterin are produced by Mtb, suggesting that it may encounter environments in which alternative respiratory systems are used (59).

It is somewhat surprising that Mtb is unable to grow anaerobically while possessing such a flexible respiratory network and fermentative pathways (51). Before concluding that hypoxia necessarily restricts bacterial growth in necrotic lesions, we should remember that respiratory limitation is a potent inducer of the growth-regulatory responses described above, and that mutants lacking DosRST or Tgs1 grow at lower oxygen tensions than wild type (45). Similarly, simply increasing CO2 concentration can dramatically potentiate Mtb growth in hypoxia (60). Thus, it remains possible that the lack of anaerobic growth thus far observed is due to a combination of regulatory effects and suboptimal culture conditions and that the hypoxic caseum could be a site of active replication.

Regardless of replication rate, respiration appears to be a primary mechanism of redox regulation in mycobacteria and the redox state of the bacterial cell has profound effects on antibiotic activity. Both too many oxidants and reductants can promote antibiotic activity in Mtb (45,61). The association between TCA activity, redox state and antibiotic-mediated killing has been demonstrated in a large number of bacterial species and systems leading to the proposal that ROS or redox-based toxicity is a common mechanism of killing (62,63). This might not be true in all systems (5,6), but the general association between redox state and antibiotic activity remains strong.

The association between respiration, redox, and antibiotic activity has significant implications for TB therapy. Different host microenvironments could induce alternative respiratory systems and influence the overall redox state of the cell. This complexity could alter drug efficacy and might even be exploited to improve therapy. An anecdotal example of this strategy is provided by the use of prodrugs that are activated in hypoxic conditions. Metronidazole (MTZ) is activated by the reduction of a nitro group under anaerobic conditions which leads to free radical mediated DNA damage (8,64). This reaction is nonproductive during aerobic conditions as the single electron-reduced species reacts with molecular oxygen. While MTZ proved too neurotoxic for long term TB treatment in humans, other nitroimidazole drug candidates (delaminid and PA-824) share the anaerobic activity of MTZ and are in late stage clinical trials (65).

Macronutrient acquisition and catabolism

All life requires nitrogen, carbon, and phosphorus. Phosphorus is generally considered to be freely available in the form of phosphate and phosphate import is required for intracellular growth (66). Nitrogen is acquired by Mtb from organic molecules, such as arginine and glutamine (reviewed in 67). These pathways and their corresponding uptake systems are both required during infection. Notably, screening conditions used for drug discovery contain similar nitrogen sources as those that are used during infection.

Despite the fact that Mtb can grow on a single carbon source, surprisingly, it needs multiple substrates during infection (67). The genome of Mtb encodes genes dedicated to the acquisition and degradation of fatty acid, cholesterol, hexose, glycerol, and amino acids. A variety of evidence suggests that most of these substrates are important for bacterial growth during infection. Both the transcriptional induction of beta oxidation genes (68) and the preferential mineralization of lipids by Mtb recovered from human lesions (69) indicates that fatty acid catabolism occurs during infection. Mtb mutants lacking the ability to acquire or degrade hexose or cholesterol are unable to maintain a persistent infection of mice (70,71). Metabolic flux modeling of Mtb growth in macrophages indicates that glycerol, likely derived from phospholipids, may also be acquired and degraded (34).

The apparent paradoxical use of multiple carbon sources is likely due to Mtb's ability to simultaneously co-catabolize multiple substrates using compartmentalized pathways (72). In many bacteria, catabolite repression systems ensure the use of a single preferred carbon source until it is exhausted. In contrast, Mtb does not have this regulatory system and optimal growth requires the co-catabolism of multiple carbon sources that independently provide glycolytic substrates and acetyl CoA (72). One can speculate that Mtb's metabolic network is adapted to growth in an environment that is replete with multiple carbon sources. Indeed, the loss of catabolite repression in *Pseudomonas aeruginosa* strains from isolated from longstanding lung infection suggests that this may be a common adaptation to chronic infection (73). The simultaneous requirement for multiple carbon sources could reflect the compartmentalization of catabolic pathways that has been described using stable isotope tracing studies under *in vitro* growth conditions (34,72). If more compartments exist in the host, different carbon sources might fulfill essential and nonredundant roles.

This compartmentalized co-catabolic metabolic model implies that even subtle differences in the relative abundance of multiple substrates could alter the metabolic state of the bacterium and influence antibiotic efficacy. The most obvious consequence of this model is that compounds targeting a specific carbon catabolic pathway may only be active under certain host microenvironments where the inhibited pathway is favored. In addition, secondar effects of metabolic pathway usage have been shown to dramatically affect drug efficacy *in vitro*. In particular, many high throughput drug screening efforts used standard laboratory media containing high concentrations of glycerol. The most active compounds found in these screens only killed bacteria that have accumulated methylglyoxal via glycerol catabolism and were inactive in infection models. Subsequent comparative whole cell screening in glycerol or acetate based media revealed that approximately 10% of all compound hits had greater activity in glycerol containing media (74).

The complexity of carbon metabolism might be exploited to enhance therapy, as we are learning that interfering with different enzymatic steps in a pathway can have significantly different effects. The cholesterol catabolic pathway provides a clear example. While the inability to import cholesterol has only a modest effect on bacterial survival in the mouse model (70), mutation of at least three different steps in the catabolic pathway result in the generation of toxic metabolic products and much more profound bacterial growth defects (75-78). Many of our most effective bacteriocidal antibiotics act by inducing similar toxic products, such as reactive oxygen species (63), truncated peptides (79), or lethal protein:DNA complexes (80). Unfortunately, these toxicities are difficult to predict *a priori*, and instead require a thorough understanding of the inhibited pathway.

Micronutrient acquisition and homeostasis

Alterations in the availability and metabolism of a variety of micronutrients during infection can also have profound effects on Mtb physiology. While Mtb is prototrophic for most vitamins and cofactors, two classes of micronutrient have received a great deal of attention, vitamin B12 and metal ions.

Mtb expresses a discrete set of B12-dependent enzymes, which are involved in ribonucleotide reduction, methionine synthesis and central carbon metabolism. While Mtb also expresses B12-independent enzymes to fulfill the first two functions, the methylmalonyl pathway that allows the assimilation of the three carbon unit, propionyl CoA, into the TCA cycle cannot be performed in the absence of B12. This pathway is particularly important for bacterial growth on cholesterol or odd-chain fatty acids, as the catabolism of these substrates can produce toxic levels of propionyl CoA (81,82).

The source of B12 during infection remains enigmatic. The Mtb genome encodes an apparently complete B12 synthetic pathway and a transporter dedicated to B12 acquisition (81,83) The bacterium does not produce its own B12 *in vitro* and, under these conditions, the cofactor-dependent pathways are completely reliant on the addition of exogenous B12 (81). It remains unclear if a currently unknown cue encountered during infection can stimulate bacterial B12 production or, alternatively, if the bacterium can scavenge this compound from the host. Regardless, the ability to experimentally alter B12 availability *in*

vitro demonstrates how the concentration of this single cofactor can profoundly alter the metabolic state of the bacterium.

A second class of critical cofactor, metal ions, represents an even more complicated homeostatic challenge for the bacterium. Like all bacteria, Mtb expresses a range of metalloproteins that rely on the coordination of divalent metal ions for their activity. The acquisition of adequate amounts of these micronutrients is essential for a variety of cellular functions, and is particularly important for respiration and oxidative stress resistance. However, the accumulation of unbound metal ions can be lethal to the cell, both directly by disrupting the proper maturation of metalloproteins and indirectly by accentuating the toxicity of reactive oxygen species (84). This latter effect is mediated by the Fenton reaction, in which the conversion of moderately toxic peroxides to extremely potent hydroxyl radicals is catalyzed by free Fe^{2+} or Cu^{2+} (84).

Due to the potent effects of alterations in metal ion concentrations, both the host and pathogen actively manipulate the availability of free metal ions. Upon recognition of infection, mammals produce a host of metal scavenging proteins that effectively starve many microorganisms for essential nutrients, such as iron and zinc (85). Successful pathogens counter this strategy by the expression of high affinity metal scavenging systems. Mtb, as a highly adapted human pathogen, effectively competes with the host for iron, through the coordinated use of free iron (86,87) and heme uptake systems (88).

While the sequestration of metals has been recognized as a mechanism of host defense for decades, more recent work suggests that mammals might also use metal toxicity to their advantage. In particular, phagocytes might actively transport metals, such as Cu^{2+} and Zn^{2+}, into the phagolysosome to enhance microbial killing (89,90). This intriguing hypothesis is supported by indirect measurements of zinc and copper concentrations in the Mtb phagosome (91) and the reported requirement for P-type ATPases that efflux metal ions for bacterial adaptation to these conditions (89,90,92). Iron chelating proteins, such as bacteroferritin and hemoglobin homologs, could provide an additional level of defense for the pathogen. However, this model should still be regarded with some skepticism, as direct measurements of metal concentrations in the mycobacterial phagosome are technically challenging and the specific roles of metal efflux systems are still being defined. For example, the CtpC protein of Mtb that was originally defined as a zinc detoxification system has more recently been found to play an additional role in metalloprotein biogenesis (93).

While our understanding of metal homeostasis in Mtb is still evolving, the potential contribution of this process to drug efficacy is undeniable. Many existing TB therapeutics induce a respiratory burst in the bacterium (62) that could be significantly potentiated by free metal ions that are capable of catalyzing the Fenton reaction. Thus, altered metal homeostasis in distinct host microenvironments might contribute to differential antibiotic efficacy, and the manipulation of Fe or Cu concentrations could be manipulated to accentuate ROS-mediated antibiotic toxicity.

Efflux systems

Drug efflux is now understood to be a major factor limiting TB chemotherapy. *M. tuberculosis* expresses efflux systems belonging to all recognized protein families, including the ATP driven ABC transporter family, and more diverse pumps driven by symport or antiport [small multidrug resistance (SMR), resistance nodulation division (RND), and major facilitator superfamily (MFS)]. The expression of these pumps is induced by drug exposure and recognized to limit the effect of a wide variety of antimycobacterial drugs during *in vitro* exposure, including INH, RIF, fluoroquinolones, aminoglycosides, and BDQ. *In vitro* studies suggest that multiple efflux systems in the MFS and ABC families may both contribute to fluoroquinolone-resistance (94).

While generally considered only in the context of drug resistance, these promiscuous efflux systems are universally produced by prokaryotes and have roles in xenobiotic resistance, secretion, and cell wall homeostasis (95). Thus, it is not surprising that efflux systems are induced to promote survival in the relatively hostile host environment, and these same pumps can act on antibiotics and alter treatment efficacy. This effect has been shown most clearly for the MFS pump encoded by the Rv1258 gene of Mtb. This protein is induced during intracellular residence and reduces the efficacy of RIF (96). Unfortunately, the lack of targeted specificity and the redundancy of these pumps in conjunction with their dynamic regulation makes their contribution to drug efficacy difficult to assess and even harder to overcome. Broad-spectrum efflux inhibitors, such as verapamil, could produce adjunctive effects and have shown some limited efficacy in the mouse model of TB. Clinical trials will be necessary to test the practicality of this strategy.

Caveats and future directions

Since the physiology of Mtb during human infection is difficult to probe directly, much of the insight discussed above is inferred from the behavior of bacteria in non-human infection models. The general caveats of these studies and strategies to relate these observations to human TB warrant discussion.

The strategies used to report on bacterial physiology *in vivo* are focused on delineating the genes or pathways that are differentially utilized between *in vitro* cultivation and infection models. Initial studies used gene expression levels as a surrogate for the importance of any specific gene (68,97). While important insights have been derived from these studies, the correlation between transcriptional induction of a gene and its contribution to the growth or survival of the bacterium is remarkably poor in bacteria including Mtb (35,98). This observation, in conjunction with the availability of high-throughput genetic methods, has made differential gene essentiality the most commonly used reporter of pathway usage *in vivo*.

A number of groups have performed genome-wide screens to identify genes that are specifically required for bacterial growth in many of the infection models described above. The methods for detecting the altered fitness of a single insertional mutant in a large pool have become very robust and quantitative, and these studies now provide virtually comprehensive data on the relative contribution of all viable loss-of-function mutants under

a specific condition. One global observation from these studies warrants mention. Despite the sensitivity of these techniques and the variety of infection models that have been investigated, a disruption of a significant fraction of the genome does not appear to cause a detectable growth defect (unpublished data). This comes as something of a surprise. The only significant niche that has contributed significantly to recent Mtb evolution is the human lung, and the reductive evolution of the *M. leprae* genome indicates that genes are rapidly lost from the genome if they do not confer a selective advantage (99). Based on this reasoning, one would predict that the vast majority of Mtb's genes contribute to bacterial fitness in at least one host microenvironment. Experience from genetic interaction studies in both Mtb and *E. coli* indicate a significant degree of functional redundancy is encoded in bacterial genomes, and Mtb encodes several large families of highly homologous genes. However, redundancy alone is unlikely to fully explain these observations. Instead, it is reasonable to conclude that a relatively large number of Mtb genes contribute to stages of the Mtb lifecycle that either affect a relatively small proportion of the bacterial population or are simply not recapitulated in our common infection models.

The potential for these large-scale studies to be confounded by environmental heterogeneity argues for methods that report on the environment of individual cells. Fluorescent transcriptional fusions have been used for this purpose (100). However, the complexity of transcriptional responses often makes these studies difficult to interpret in the absence of direct reporters of metabolite levels or physiological state. Tools to measure critical aspects of bacterial metabolism, such as redox state and ATP levels, have been developed and promise to close this critical gap (101-103). The application of these new methods in models that include nonhuman primates will be necessary to truly understand bacterial physiology during human infection.

Translating insight into drugs

Target-based antibacterial drug discovery

The advent of whole genome sequencing and high-throughput genetic analysis has provided a wealth of potential drug targets over the past 15 years. However, translating this information into effective inhibitors remains a challenge. The experience of GlaxoSmithKline (GSK) is an informative example. This company pursued 70 high-throughput screening (HTS) campaigns against a variety of individual bacterial targets and complete biochemical pathways using an extensive chemical collection (104). This effort yielded only five lead compounds with whole cell activity. This experience was mirrored at other organizations with the more than 125 antibacterial screens on 60 individual targets yielding no candidates for further development (105). These disappointing results have been extensively reviewed elsewhere (104,105) and are supported by a smaller sample of experiences from Mtb. For example, the isocitrate lyases are key glyoxylate-shunt pathway enzymes that are essential for intracellular growth (106) and are absent from in mammalian cells. Despite the biological attractiveness of this target and a great deal of effort, effective inhibitors have not been described and the isocitrate lyases have been deemed ‘undruggable’ (107).

As a result of these experiences, most current drug development efforts begin with whole cell ('phenotypic') screening to identify lead compounds that show some activity against intact cells, only to subsequently discover their mechanism of action. Given these realities of drug development, how can we translate the knowledge of Mtb physiology described above into more effective therapies? Two very different approaches are currently being pursued to accomplish this goal.

Screen for compounds that are active under conditions that mimic infection Since most of our current drugs were optimized to kill rapidly growing bacteria in axenic culture, the relative inefficacy of TB drugs during infection might be due to the preselection of compounds that target a metabolic state that is not reflective of the *in vivo* condition. Taking advantage of what we know about Mtb metabolism during infection might lead to screens for more effective compounds. Indeed, PZA, among the most potent sterilizing TB drugs, has little activity under standard culture conditions and would likely be discarded in modern drug development programs. However, PZA is bacteriocidal in the low pH conditions like those encountered during infection. This example supports the concept of performing drug screening under more "host like" conditions.

Unfortunately, the complexity and heterogeneity of stresses experienced by Mtb during infection (108-118) makes the design of relevant screening conditions a major challenge. One approach is to combine multiple conditions (119). They performed HTS under a combined stress condition consisting of hypoxia, low pH, NO stress and short chain fatty acids as a carbon source. Among the compounds they identified was oxyphenbutazone (OPB), a commonly used nonsteroidal anti-inflammatory drug, which was transformed by these conditions into a metabolite that effectively killed non-replicating Mtb (119). The active anti-mycobacterial form of OPB, 4-OH-OPB, is produced under mild acid treatment and upon exposure to RNIs. 4-OH-OBP accumulates rapidly in non-replicating Mtb and depletes flavins, compromising many enzymatic reactions. OPB is not usable in the mouse model due to specific drug metabolism in this species; however, the current use of OBP in clinical settings will facilitate trials that could promote the inclusion of this NSAID with current treatment (119). While the ultimate utility of OBP in TB treatment remains to be determined, this example suggests not only that it is possible to identify compounds that effectively kill nonreplicating bacteria, but also that small molecules have vastly different effects under different conditions.

To mitigate the uncertainty in the design of physiologically relevant *in vitro* screening conditions, some have turned to screening directly in simple infection models. Infection of a macrophage cell line with mycobacteria constitutively expressing green fluorescent protein (GFP) formed the basis for the first Mtb screen of this kind (33). Using quantitative fluorescence microscopy, the authors were able to quantify bacterial replication during treatment with a small molecule library and identified many active compounds including dinitrobenzamide derivatives (DNB). DNB derivatives DNB1 and DNB2 are particularly potent with MICs of 200 nM against Mtb. Further testing of the DNB series revealed that the nitro groups necessary for anitmycobacterial action are rapidly reduced by mammalian liver enzymes (33), potentially limiting the potency of these compounds *in vivo*. A modification of this intracellular survival screen to include an initial *in vitro* test of mycobacteria survival

using a resazurin reduction assay, then an intracellular GFP-based survival screen with selected compounds unveiled four compounds with apparent anti-mycobacterial activity (120). Even more complex models, such as intact zebrafish embryos (32), have been proposed or screening.

Despite these early successes, it remains unclear whether simple infection models such as these mimic the environment(s) that could harbor the most relevant populations of bacteria *in vivo*. The relative value of each model will become clear as compounds derived from these screens are developed and tested in additional systems.

Pathway-directed whole cell screens

As described above, distinct biochemical pathways are used during infection. Would targeting these pathways, using genetically engineered strains that are specifically sensitized, yield more efficacious drugs? In many organisms including Mtb, reducing the expression level of an essential gene will increase a cell's sensitivity to inhibitors that act, directly or indirectly, on the same biochemical pathway (121). This differential screening approach has several advantages. Most notably, small molecules can be directly identified that act on intact cells and are likely to target a pathway that is known to be important for infection. In addition, the increased sensitivity of hypomorphic strains might make it possible to identify lead compounds with weak activity that would not be apparent in a standard whole cell screen. This general strategy has been used in a variety of systems, ranging from zebrafish (122) to bacteria (123-127), and has been successful in identifying a number of new antifungal leads (128).

This approach has been applied to mycobacteria, a feat made possible in large part by exquisitely tunable tetracycline-based conditional gene expression systems (129,130). Abrahams *et al*. (123) probed three distinct pathways with this approach, pantothenate and lysine synthesis, and the glyoxylate shunt; through the regulated expression of pantothenate synthetase (panC), diaminopimelate decarboxylase (lysA), and isocitrate lyase (icl1). Novel inhibitors of PanC were identified. In addition, flavones were isolated from this screen did not inhibit the biochemcial activity of PanC *in vitro* but were still preferentially inhibited the growth of PanC hypomorphic strains, implying that these compounds act on an alternate target(s) in this pathway. Thus, this approach appears to enable the identification of compounds that act directly targets and indirectly against components of a specific pathway.

Other types of genetically engineered reporter strains can be used to identify whole cell-active compounds that inhibit specific cellular functions thought to be important during infection. For example, Mtb is exposed to an acidic environment in the phagosome and pH sensitive mutants are attenuated for virulence (111, 131). To identify pathways required for intra-bacterial pH homeostasis, a pH-sensitive, ratiometric GFP was adapted to Mtb (131). The fluorescent bacilli were incubated with compounds from a natural product library under acidic conditions to find those that killed by disrupting pH homeostasis. In this strain, PZA in acidic culture conditions directly lowered intrabacterial pH (131), providing information about the mechanism of cidal activity of PZA and suggesting that this screen could be used to isolate compounds with similar activity. One of the validated hits from this screen, agrimophol, is bactericidal at both acidic and neutral pH, suggesting multiple possible

mechanisms of action. The increasing ease with which hypomorphic mutants and other reporter strains can be developed suggests that these types of targeted whole cell screens will become ever more useful.

In sum, the integration of biological insight and drug development is not straightforward. The future development of additional antibacterial agents effective against the many metabolic states of Mtb during human infection requires integration of acquired knowledge about the localized microenvironments encountered during infection, and the design of drug development paradigms to target the specific combination of pathways that are required in each. The value of each approach toward this integration will become clear only when compounds are identified and optimized such that they can be tested in preclinical and clinical studies.

Can we exploit drug synergies to improve therapy?

In a perfect world, a single drug could be used to rapidly eradicate Mtb from all host microenvironments. In reality, multidrug therapy is universally used to treat TB, and it is clear that the effect of a regimen is not merely the sum of its parts. Instead, these drugs interact with each other and the host in ways that can either accentuate or minimize their ultimate effect. Current regimens were assembled from a small number of existing drugs and designed based on clinical experience. In the next section, we will explore whether drug synergies can be more actively identified and exploited to improve therapy. Three types of synergy will be discussed here: ‘classical synergy’ where compounds inhibit functionally redundant bacterial targets, ‘physiological synergy’ where compounds preferentially act on distinct metabolic states of the bacterium, and ‘host-pathogen synergy’ in which a host-directed drug accentuates the activity of a standard anti-bacterial (*Fig. 2*).

Classical synergy

Synergy is a fundamental concept in genetics, as the effect of inhibiting most genes can be buffered by the action of others. This effect can be explained by a variety of mechanisms, including the presence of chaperones that promote the function of inhibited genes, partially redundant enzymatic activities, or bypass pathways. Instead of being a rare curiosity, genome-wide studies in yeast indicate that ~40% of genes are functionally buffered by at least one other (132). The practical result of this functional architecture is that the effect of inhibiting a gene product can often be potentiated by the simultaneous inhibition of a buffering activity.

This effect has been exploited in a handful of cases to potentiate antibacterial therapy. Most notably, the mixture of the bacteriostatic folate inhibitors trimethoprim and sulfamethoxazole (TMP-SMX) produces a synergistic bacteriocidal effect, and these drugs are often co-administered. In fact, sensitivity to this combination has been demonstrated for multiple clinical isolates of Mtb despite the low activity of TMP alone (133). While reports reveal widely varying responses to TMP and SMX, alone and in combination (134), some drug resistant strains appear to be susceptible to TMP –SMX.

In another example of this classical type of synergy, the addition of a β-lactamase inhibitor, such as clavulanate, can potentiate the activity of β-lactam antibiotics by preventing their inactivation. The combination of clavulanate and meropenem is highly bactericidal *in vitro* against Mtb, and this combination proved efficacious in accelerating the clearance of bacteria from sputum when combined with linezolid-containing regimens for MDR and XDR-TB patients (135).

While these individual synergies may prove useful, strategies to directly identify new synergistic compounds could be much more exciting in the long term. A concerted effort to identify these synergies in Mtb has not been reported, but directed phenotypic screens have been successfully used to search or useful synergies between antimalarial compounds. For example, chloroquine (CQ) resistance in *Plasmodium falciparum*, based on drug efflux, resulted in the removal of this antimalarial from front-line chemotherapy in the 1990s (136). A simple combination screen utilizing fluorescently tagged CQ and a small molecule library of inhibitors yielded chemosensitizing agents that enable CQ uptake by blocking drug efflux pumps and by facilitating the entry through an unknown mechanism, enabling the necessary intracellular accumulation of the compound for killing (136).

Both chemical and genetic approaches have been used to exploit synergies in staphylococci. Methicillin-resistant strains of *S. aureus* (MRSA) rely on an alternative peptidoglycan transpeptidase activity to grow in the presence of β-lactam antibiotics. To identify strategies to re-sensitize these strains to β-lactams, chemical screens have been used to identify a number of PG inhibitors that act synergistically with β-lactams (137). A more biologically informative approach to this problem employed a genetic screen using an antisense library of essential gene knockdowns in MRSA (138). Inhibition of several components of the peptidoglycan synthetic pathway, as well as unexpected components of the cell division machinery including FtsZ, was found to increase susceptibility to oxacillin. Since such a large number of sensitizing mutations were found using this high-throughput approach, tool compounds were available to test the efficacy of synergistic therapy. When combined with oxacillin, the FtsZ inhibitor PC190723 displayed strong synergy *in vitro* and *in vivo* using a murine model of deep tissue MRSA infection. The identification of this type of synergistic interaction could overcome the phenotypic buffering inherent in all biological systems, which likely limits the efficacy of many, if not most, antibiotics.

Synergistic targeting of multiple physiological states

Using drugs that target distinct metabolic states likely underlies the increased efficacy of combination therapy (139,140). INH is thought to rapidly kill replicating bacteria, while RIF and PZA target slowly- or non-replicating populations. Entirely new regimens that could overcome resistance to the standard drug regimen are under development. By defining the mechanism of action of new drugs, in conjunction with a more detailed understanding of diverse bacterial physiologic states*in vivo*, it may be possible rationally design regimens that are even more effective.

An early example of this strategy involves an new regimen that includes the bicyclic nitroimidazole compound, PA-824, that is active against both replicating and oxygen-starved nonreplicating bacteria (141). A combination of PA-824, moxifloxicain, and PZA

significantly shortened treatment in mice, when compared to the standard RIF/INH/PZA combination (142). The combination was validated in an early bactericidal activity (EBA) study in human patient sputum that compared BDQ, BDQ-PZA, PA-824-PZA, BDQ-PA-824, PA-824-MXF-PZA, or standard therapy (143). The 14-day EBA of PA-824-MXF-PZA, was comparable with standard treatment, providing initial evidence of clinical efficacy. It remains unclear whether this type of EBA study truly samples the diversity of bacterial states that need to be eradicated to cure an infection. Only longer term clinical trials that using relapse as an endpoint will truly determine if such a new regimen is effective against the bacterial subpopulations that are the most relevant to bacterial persistence during drug therapy. However, the generation of regimens that target distinct bacterial states promises both to improve therapy and could be used to probe the bacterial subpopulations that are critical for cure in humans.

Host/bacteria synergy

Mtb has co-evolved with the human host to exist in an uneasy homeostasis. The majority of individuals can contain the infection in an asymptomatic state. In fact, recent evidence from nonhuman primate models (18) confirms the inference from human autopsy studies (16) that many human TB lesions are effectively sterilized by the immune response. The ability of most individuals to resist Mtb infection implies that it might be possible to coax the immune system to protect those individuals that are more prone to disease. This strategy to inhibit pathogen survival by modifying the host pathways has proven useful in the treatment of viral pathogens such as hepatitis C (HCV). Treatment with Peginterferon plus ribavirin is standard for chronic HCV infection, because this combination of host-directed and virus-directed inhibitors enhances viral clearance (144). However, this approach is a relatively untapped therapeutic avenue for bacterial infections.

The possibility of host-directed therapy (HDT) for TB is the topic of a great deal of current interest and a wide variety of compounds have been proposed for investigation, including modulators of immunity, ion channels, kinase cascades, inflammation, autophagy, and lipid metabolism (145-149). This topic has been the subject of several excellent reviews. However, it seems unlikely that any HDT will be used in the absence of direct antimicrobial therapy, and in the context of this review, we will concentrate on the potential for synergy between host- and bacteria-targeted compounds. In particular, HDTs could be exploited to increase antibiotic penetration in lesions, to change the local environment to enhance antibiotic action, and to accelerate the sterilizing activity of the host immune response. Below, we discuss the general mechanisms by which HDTs could enhance therapy, and then relate these mechanisms with examples of possible strategies to exploit host-pathogen synergies.

Enhancing antibiotic exposure

While the pharmacokinetic behavior of drugs is generally measured in the plasma, antibiotic activity depends upon local concentration. If a therapeutic concentration is not reached at the site of infection, the bacteria will be exposed to sub-inhibitory doses that are both ineffective and can select for drug-resistant variants (150). Active human TB disease manifests with a variety of lesion types including activated cellular granuloma, necrotic and caseous

granulomas and fibrotic cavities (151). During treatment, administered compounds need to penetrate these variably vascularized tissues to reach the bacilli and the ability to do so likely varies according to the chemical composition of each inhibitor. Early studies to measure drug penetration of TB lesions at the macroscopic level concluded the granuloma did not limit exposure to INH (69). However, contemporary high resolution studies of lesional pharmacokinetics present a starkly different picture.

Many drugs, such as RIF, INH, and PZA, are present in lower abundance in tissue than in plasma (151). MXF, a very effective second line agent, exhibits a significantly higher tissue to plasma concentration ratio, suggesting that the effective penetration of this compound into infected environments may account for some of its success as an anti-mycobacterial. Indeed, the use of Matric-assisted laser desorption/ionization-multi-reaction monitoring-mass spectrometry imaging (MALDI-MRM-MSI) which is used for spatially resolving drug concentrations at a microscopic level within tissue, revealed accumulation of MXF in granulomas in infected rabbit lungs (152). Even within granulomas, variation in concentration was observed. Cellular areas had the highest levels while areas of necrotic caseum had the lowest. Differential penetration is a property shared by RIF, which accumulates in uninfected tissue but is reduced in granulomas (4).

The concentration of drugs in different anatomical locations might also provide a partial explanation for the remarkably high rates at which drug resistant isolates emerge during combination therapy. If bacteria were exposed to all four drugs in the standard regimen simultaneously, the emergence of a clone spontaneously resistant to all four should be vanishingly rare. Unfortunately, MDR strains emerge even in populations where directly observed therapy programs ensure treatment compliance. The uneven distribution of these drugs in a single lesion suggest that individual bacteria may not be exposed to inhibitory concentrations of all four drugs, allowing the sequential selection of resistance to each drug.

These observations indicate antibiotic penetration into the TB lesion could be a significant limitation to therapy. Fortunately, several of the immunological mediators involved in the formation and maintenance of the granuloma are known (see below), suggesting strategies to disrupt the structures that limit antibiotic exposure.

Altering bacterial state

As discussed above, a primary factor that is thought to limit antibiotic efficacy *in vivo* is the altered growth rate and physiological state of the bacterium during infection. Reversing these metabolic adaptations can dramatically enhance antibacterial efficacy (45). As the metabolism of the pathogen is largely dictated by the local host microenvironment and immune pressures, it is reasonable to assume that the modulation of host immunity could likewise return the bacterium to a relatively drug sensitive state that resembles the *in vitro* conditions under which our antibiotics were initially optimized. Some evidence in favor of this strategy can be gleaned from animal models. For example, treatment of immunocompromised gamma interferon-deficient mice results in faster initial clearance of bacteria than animals with an intact immune system (153). This represents a useful proof of the concept. However, it is unlikely that overt immunosuppression will be beneficial.

Immunosuppression of previously antibiotic-treated animals can induce a recurrence of disease, indicating that the immune system is important for preventing relapse (154). The high rate of TB treatment failure in HIV-infected humans is also consistent with an important role for host immunity in the ultimate eradication of Mtb infection (155). Thus, specific modulation of immunity without overt immunosuppression may be necessary to augment bacterial clearance by antibiotics without inhibiting the ultimate eradication of the pathogen.

Examples of host-pathogen synergy

While many HDTs have been proposed based on our general understanding of disease, only a few have been investigated in the context of simultaneous antimicrobial therapy. Most of the compounds that have been tested thus far are immunomodulators that can act to increase bacterial killing and/or ameliorate pathology.

The cytokine tumor necrosis factor (TNF) is critical for granuloma formation in response to a wide variety of insults, including Mtb infection (156,157). Inhibition of TNF with the small molecule phosphodiesterase IV inhibitor, CC-3052 (158), or the soluble TNF receptor, etanercept (159), leads to the disruption of granuloma architecture in murine, rabbit, and non-human primate models of TB, an activity that could explain the ability of TNF antagonists to modestly increase the efficacy of antimicrobial therapy and decrease relapse in rabbits (158). The increased rate of bacterial clearance from sputum in HIV/TB coinfected patients treated with etanercept in conjunction with standard therapy indicates a similar effect in humans (160). It is currently unclear if the apparent synergy with antimicrobials is a result of alterations in drug access or microbial metabolism, but this important question could be addressed by quantifying the lesional pharmacokinetics in treated animals.

Even commonly used nonsteroidal anti-inflammatories (NSAID) have been found to enhance antibiotic therapy in animal models. Diclofenac synergizes with streptomycin therapy (161), and both aspirin and ibuprofen enhance the bacteriocidal effect of PZA (162). The mechanisms that underlie these effects are unknown and involve more than just enhancement of antibiotic action, as ibuprofen or aspirin alone can reduce bacterial burden in highly susceptible mice (163). The primary effects of these drugs on eicosanoid balances represents a likely mechanism, as low doses of aspirin, which are not generally anti-inflammatory but can induce lipoxin production, are sufficient for synergy (162).

In addition to these attempts to accentuate bacterial killing, similar immunomodulators are used to alleviate acute pathology. For example, corticosteroid therapy is often used in the context of TB meningitis. An additional possible target for intervention is the long-term lung damage, fibrosis, and pulmonary hypertension caused by previous TB disease. Lung function has been shown to decrease with each episode of TB, with the percentage of subjects with chronic airflow impairment increasing from 18.4% in those with one episode, to 27.1% and 35.2% in those with two or three episodes of tuberculosis (164). In fact, if the burden of well-treated TB is calculated in terms of disability adjusted life years (DALY), chronic pulmonary impairment accounts for 75% of the burden, with years of life lost and

acute disease accounting for only 25%. The extent of long-term lung damage is at least in part determined by host genetics, as a polymorphism that causes high production of the inflammatory cytokine IL-1β is associated with persistent disease, as measured by quantitative tomography (165). A greater understanding of the immunological mediators that lead to these pathologies should indicate additional targets that can be modulated to prevent long-term pulmonary impairment.

These initial forays into host/pathogen synergies may or may not translate into useful new therapies. However, a wide variety of immunomodulators are currently in clinical trials or approved for other indications, which could allow the clinical utility of this approach to be tested relatively rapidly if the resources necessary for these studies were available.

Summary and future directions

The advent of tuberculosis chemotherapy six decades ago was a miraculous success, turning a disease with a 50% mortality rate into an almost uniformly curable condition. In conjunction with rigorous public health measures, these TB treatments have largely erased this disease from the developed world. However, TB therapy has changed little over the past decades. The long duration of the antibiotic regimen, in conjunction with drug resistance and co-morbidities such as HIV and diabetes, has greatly hampered our ability to control TB throughout much of the world. While the lack of continued drug development efforts over past decades has certainly contributed to the stagnation in TB therapeutic strategies, recent intensive effort has also failed to produce radically more effective drugs. It could be argued that our inability to improve TB therapy is primarily due to our lack of understanding the fundamental biology that underlies TB pathogenesis and antibiotic efficacy *in vivo*.

While TB drug design lagged for several decades, this period also represented a renaissance in TB biology that witnessed the development of sophisticated mycobacterial genetics, a wealth of animal models, and fundamental immunological insight. If we are to take practical advantage of this information to improve therapy we must close the gaps in our biological understanding of the disease and translate this insight into new drugs

We are challenged to understand the host and pathogen physiological factors that determine antibiotic efficacy *in vivo*. As described above, this work is well underway. We remain limited, as our most experimentally tractable animal model, the mouse, might not reproduce the wide variety of disease states observed in human patients. This single animal model produced the vast majority of biological insight into TB biology, and represents the most practical preclinical model for new therapies. However, significant immunological differences exist between rodents and primates (166, 167), leading some researchers to advocate against testing new drugs in small animal preclinical models of TB. Others argue that the activity of our current drugs in the standard C57BL/6 mouse model largely predicts their sterilizing activity in humans (168), and while the model is not perfect, it is predictive in the context of antibiotic development. However, a more nuanced view could incorporate the diversity of disease states that are observed in different strains of mice. While no single animal produces the diversity of disease observed in primates, different genotypes develop a wide variety of pathologies. A dedicated effort to compare the immunological environment

and bacterial physiological state in diverse animal models with those observed in lesions of humans or non-human primates could transform our ability to mechanistically dissect disease progression and test drug activity in relevant host microenvironments.

While challenges exist, these should not dampen our optimism. In virtually any other realm of medicine, increased biological understanding of a disease affords additional therapeutic opportunities. There is no reason that antibacterial development should not follow this same path. Many prospects exist for novel chemotherapeutics that will improve TB drug therapy.

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References

1. WHO. Global tuberculosis report 2013. World Health Organization; Geneva: 2013.
2. Tuberculosis), J. J. E. t. E. Creation of DOTS.
3. Aparna SB, Reddy VC, Gokhale S, Moorthy KV. In vitro drug resistance and response to therapy in pulmonary tuberculosis patients under a DOTS programme in south India. Trans R Soc Trop Med Hyg. 2009; 103:564–570. [PubMed: 19243801]
4. Dartois V. The path of anti-tuberculosis drugs: from blood to lesions to mycobacterial cells. Nat Rev Microbiol. 2014; 12:159–167. [PubMed: 24487820]
5. Aldridge BB, Fern M, Fernandez-Suarez M, ez-Suarez, Heller D, Ambravaneswaran V, Irimia D, Toner M, Fortune SM. Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility. Science (New York, N.Y.). 2012; 335:100–104.
6. Wakamoto Y, Dhar N, Chait R, Schneider K, Signorino-Gelo F, Leibler S, McKinney JD. Dynamic persistence of antibiotic-stressed mycobacteria. Science (New York, N.Y.). 2013; 339:91–95.
7. Rittershaus ES, Baek SH, Sassetti CM. The normalcy of dormancy: common themes in microbial quiescence. Cell Host Microbe. 2013; 13:643–651. [PubMed: 23768489]
8. Wayne LG, Sramek H. Metronidazole is bactericidal to dormant cells of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1994
9. Paramasivan CN, Sulochana S, Kubendiran G, Venkatesan P, Mitchison DA. Bactericidal action of gatifloxacin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2005; 49:627–631. [PubMed: 15673743]
10. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2003; 7:6–21.
11. YEAGER RL, MUNROE WG, DESSAU FI. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. American review of tuberculosis. 1952; 65:523–546. [PubMed: 14924175]
12. Zhang Y, Wade M, Scorpio A. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. Journal of Antimicrobial Chemotherapy. 2003; 52:790–795. [PubMed: 14563891]
13. Zimhony O, Vilchéze C, Arai M, Welch JT, Jacobs WR. Pyrazinoic acid and its n-propyl ester inhibit fatty acid synthase type I in replicating tubercle bacilli. Antimicrob Agents Chemother. 2007; 51:752–754. [PubMed: 17101678]
14. Ngo SC, Zimhony O, Chung WJ, Sayahi H, Jacobs WR, Welch JT. Inhibition of isolated Mycobacterium tuberculosis fatty acid synthase I by pyrazinamide analogs. Antimicrob Agents Chemother. 2007; 51:2430–2435. [PubMed: 17485499]

15. Shi W, Zhang X, Jiang X, Yuan H, Lee JS, C. E. Wang H, Zhang W, Zhang Y. Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis. Science (New York, N.Y.). 2011; 333:1630–1632.
16. Canetti, G. The tubercle bacillus in the pulmonary lesion of man; histobacteriology and its bearing on the therapy of pulmonary tuberculosis, [American rev. ed. Springer Pub. Co.; New York: 1955.
17. Wolf AJ, Linas B, Trevejo-Nunez GJ, Kincaid E, Tamura T, Takatsu K, Ernst JD. Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. J Immunol. 2007; 179:2509–2519. [PubMed: 17675513]
18. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, Sacchettini J, Fortune SM, Flynn JL. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nature Medicine. 2014; 20:75–79.
19. North RJ, Jung YJ. Immunity to tuberculosis. Annu Rev Immunol. 2004; 22:599–623. [PubMed: 15032590]
20. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. The Journal of experimental medicine. 1993; 178:2249–2254. [PubMed: 7504064]
21. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. The Journal of experimental medicine. 1993; 178:2243–2247. [PubMed: 8245795]
22. Behar SM, Dascher CC, Grusby MJ, Wang CR, Brenner MB. Susceptibility of mice deficient in CD1D or TAP1 to infection with Mycobacterium tuberculosis. The Journal of experimental medicine. 1999; 189:1973–1980. [PubMed: 10377193]
23. McCune RM Jr. Tompsett R. Fate of Mycobacterium tuberculosis in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli in the tissues despite prolonged antimicrobial therapy. The Journal of experimental medicine. 1956; 104:737–762. [PubMed: 13367341]
24. Munoz-Elias EJ, Timm J, Botha T, Chan WT, Gomez JE, McKinney JD. Replication dynamics of Mycobacterium tuberculosis in chronically infected mice. Infection and Immunity. 2005; 73:546–551. [PubMed: 15618194]
25. Tsai MC, Chakravarty S, Zhu G, Xu J, Tanaka K, Koch C, Tufariello J, Flynn J, Chan J. Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. Cell Microbiol. 2006; 8:218–232. [PubMed: 16441433]
26. Via LE, Lin PL, Ray SM, Carrillo J, Allen SS, Eum SY, Taylor K, Klein E, Manjunatha U, Gonzales J, Lee EG, Park SK, Raleigh JA, Cho SN, McMurray DN, Flynn JL, Barry CE 3rd. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. Infection and Immunity. 2008; 76:2333–2340. [PubMed: 18347040]
27. Hunter RL. Pathology of post primary tuberculosis of the lung: an illustrated critical review. Tuberculosis (Edinb). 2011; 91:497–509. [PubMed: 21733755]
28. Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. Nature reviews. Immunology. 2012; 12:352–366.
29. Andries K, Gevers T, Lounis N. Bactericidal potencies of new regimens are not predictive of their sterilizing potencies in a murine model of tuberculosis. Antimicrob Agents Chemother. 2010; 54:4540–4544. [PubMed: 20713662]
30. Eum SY, Kong JH, Hong MS, Lee YJ, Kim JH, Hwang SH, Cho SN, Via LE, Barry CE 3rd. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. Chest. 2010; 137:122–128. [PubMed: 19749004]
31. Brodin P, Poquet Y, Levillain F, Peguillet I, Larrouy-Maumus G, Gilleron M, Ewann F, Christophe T, Fenistein D, Jang J, Jang MS, Park SJ, Rauzier J, Carralot JP, Shrimpton R, Genovesio A, Gonzalo-Asensio JA, Puzo G, Martin C, Brosch R, Stewart GR, Gicquel B, Neyrolles O. High content phenotypic cell-based visual screen identifies Mycobacterium tuberculosis acyltrehalose-containing glycolipids involved in phagosome remodeling. PLoS Pathog. 2010; 6:e1001100. [PubMed: 20844580]

32. Carvalho R, de Sonneville J, Stockhammer OW, Savage ND, Veneman WJ, Ottenhoff TH, Dirks RP, Meijer AH, Spaink HP. A high-throughput screen for tuberculosis progression. PLoS One. 2011; 6:e16779. [PubMed: 21390204]
33. Christophe T, Jackson M, Jeon HK, Fenistein D, Contreras-Dominguez M, Kim J, Genovesio A, Carralot J, Ewann F, Kim EH, Lee SY, Kang S, Seo MJ, Park EJ, Skovierová H, Pham H, Riccardi G, Nam JY, Marsollier L, Kempf M, Joly-Guillou M, Oh T, Shin WK, No Z, Nehrbass U, Rol, Brosch R, Brosch, Cole ST, Brodin P. High content screening identifies decaprenyl-phosphoribose 2' epimerase as a target for intracellular antimycobacterial inhibitors. PLoS Pathog. 2009; 5:e1000645. [PubMed: 19876393]
34. Beste DJ, Nöh K, Niedenführ S, Mendum TA, Hawkins ND, Ward JL, Beale MH, Wiechert W, McFadden J. 13C-flux spectral analysis of host-pathogen metabolism reveals a mixed diet for intracellular Mycobacterium tuberculosis. Chemistry & Biology. 2013; 20:1012–1021. [PubMed: 23911587]
35. Rengarajan J, Bloom BR, Rubin EJ. Genome-wide requirements for Mycobacterium tuberculosis adaptation and survival in macrophages. Proc Natl Acad Sci U S A. 2005; 102:8327–8332. [PubMed: 15928073]
36. Berg RD, Ramakrishnan L. Insights into tuberculosis from the zebrafish model. Trends Mol Med. 2012; 18:689–690. [PubMed: 23084762]
37. Apt A, Kramnik I. Man and mouse TB: contradictions and solutions. Tuberculosis (Edinb). 2009; 89:195–198. [PubMed: 19345146]
38. Manabe YC, Kesavan AK, Lopez-Molina J, Hatem CL, Brooks M, Fujiwara R, Hochstein K, Pitt ML, Tufariello J, Chan J, McMurray DN, Bishai WR, Dannenberg AM Jr. Mendez S. The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. Tuberculosis (Edinb). 2008; 88:187–196. [PubMed: 18068491]
39. Kaushal D, Mehra S, Didier PJ, Lackner AA. The non-human primate model of tuberculosis. J Med Primatol. 2012; 41:191–201. [PubMed: 22429048]
40. Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, Higgins DE, Daly MJ, Bloom BR, Kramnik I. Ipr1 gene mediates innate immunity to tuberculosis. Nature. 2005; 434:767–772. [PubMed: 15815631]
41. Driver ER, Ryan GJ, Hoff DR, Irwin SM, Basaraba RJ, Kramnik I, Lenaerts AJ. Evaluation of a Mouse Model of Necrotic Granuloma Formation Using C3HeB/FeJ Mice for Testing of Drugs against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2012; 56:3181–3195. [PubMed: 22470120]
42. Reece ST, Loddenkemper C, Askew DJ, Zedler U, Schommer-Leitner S, Stein M, Mir FA, Dorhoi A, Mollenkopf HJ, Silverman GA, Kaufmann SH. Serine protease activity contributes to control of Mycobacterium tuberculosis in hypoxic lung granulomas in mice. J Clin Invest. 2010; 120:3365–3376. [PubMed: 20679732]
43. Gill WP, Harik NS, Whiddon MR, Liao RP, Mittler JE, Sherman DR. A replication clock for Mycobacterium tuberculosis. Nature Medicine. 2009; 15:211–214.
44. Mitchison DA. The search for new sterilizing anti-tuberculosis drugs. Front Biosci. 2004; 9:1059–1072. [PubMed: 14977529]
45. Baek SH, Li AH, Sassetti CM. Metabolic regulation of mycobacterial growth and antibiotic sensitivity. PLoS biology. 2011; 9:e1001065. [PubMed: 21629732]
46. Nguyen D, Joshi-Datar A, Lepine F, Bauerle E, Olakanmi O, Beer K, McKay G, Siehnel R, Schafhauser J, Wang Y, Britigan BE, Singh PK. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science. 2011; 334:982–986. [PubMed: 22096200]
47. Primm TP, Andersen SJ, Mizrahi V, Avarbock D, Rubin H, C. E. The stringent response of Mycobacterium tuberculosis is required for long-term survival. J Bacteriol. 2000; 182:4889–4898. [PubMed: 10940033]
48. Weiss LA, Stallings CL. Essential roles for Mycobacterium tuberculosis Rel beyond the production of (p)ppGpp. J Bacteriol. 2013; 195:5629–5638. [PubMed: 24123821]

49. Mitchison D, Davies G. The chemotherapy of tuberculosis: past, present and future. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2012; 16:724–732.
50. Small JL, Park SW, Kana BD, Ioerger TR, Sacchettini JC, Ehrt S. Perturbation of cytochrome c maturation reveals adaptability of the respiratory chain in Mycobacterium tuberculosis. mBio. 2013; 4:e00475–00413. [PubMed: 24045640]
51. Watanabe S, Zimmermann M, Goodwin MB, Sauer U, Barry CE 3rd, Boshoff HI. Fumarate reductase activity maintains an energized membrane in anaerobic Mycobacterium tuberculosis. PLoS Pathog. 2011; 7:e1002287. [PubMed: 21998585]
52. Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR, Schoolnik GK. Inhibition of respiration by nitric oxide induces a Mycobacterium tuberculosis dormancy program. The Journal of experimental medicine. 2003; 198:705–713. [PubMed: 12953092]
53. Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D, Kaufmann SH. Unique Transcriptome Signature of Mycobacterium tuberculosis in Pulmonary Tuberculosis. Infection and Immunity. 2006; 74:1233–1242. [PubMed: 16428773]
54. Tan MP, Sequeira P, Lin WW, Phong WY, Cliff P, Ng SH, Lee BH, Camacho L, Schnappinger D, Ehrt S, Dick T, Pethe K, Alonso S. Nitrate respiration protects hypoxic Mycobacterium tuberculosis against acid- and reactive nitrogen species stresses. PLoS One. 2010; 5:e13356. [PubMed: 21048946]
55. Wayne LG, Hayes LG. Nitrate reduction as a marker for hypoxic shiftdown of Mycobacterium tuberculosis. Tubercle and Lung Disease. 1998; 79:127–132. [PubMed: 10645451]
56. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG, Ye JD, Pati M, Vercruysse M, Ralifo PS, Allison KR, Khalil AS, Ting AY, Walker GC, Collins JJ. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci U S A. 2014; 111:E2100–2109. [PubMed: 24803433]
57. Winter SE, Lopez CA, Baumler AJ. The dynamics of gut-associated microbial communities during inflammation. EMBO Rep. 2013; 14:319–327. [PubMed: 23478337]
58. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Baumler AJ. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science. 2013; 339:708–711. [PubMed: 23393266]
59. Williams MJ, Kana BD, Mizrahi V. Functional analysis of molybdopterin biosynthesis in mycobacteria identifies a fused molybdopterin synthase in Mycobacterium tuberculosis. J Bacteriol. 2011; 193:98–106. [PubMed: 20971904]
60. Gazdik MA, McDonough KA. Identification of cyclic AMP-regulated genes in Mycobacterium tuberculosis complex bacteria under low-oxygen conditions. J Bacteriol. 2005; 187:2681–2692. [PubMed: 15805514]
61. Bhaskar A, Chawla M, Mehta M, Parikh P, Chandra P, Bhave D, Kumar D, Carroll KS, Singh A. Reengineering redox sensitive GFP to measure mycothiol redox potential of Mycobacterium tuberculosis during infection. PLoS Pathog. 2014; 10:e1003902. [PubMed: 24497832]
62. Grant SS, Kaufmann BB, Chand NS, Haseley N, Hung DT. Eradication of bacterial persisters with antibiotic-generated hydroxyl radicals. Proc Natl Acad Sci U S A. 2012; 109:12147–12152. [PubMed: 22778419]
63. Dwyer DJ, Kohanski MA, Collins JJ. Role of reactive oxygen species in antibiotic action and resistance. Curr Opin Microbiol. 2009; 12:482–489. [PubMed: 19647477]
64. Lin PL, Dartois V, Johnston PJ, Janssen C, Via L, Goodwin MB, Klein E, Barry CE 3rd, Flynn JL. Metronidazole prevents reactivation of latent Mycobacterium tuberculosis infection in macaques. Proc Natl Acad Sci U S A. 2012; 109:14188–14193. [PubMed: 22826237]
65. Carroll MW, Jeon D, Mountz JM, Lee JD, Jeong YJ, Zia N, Lee M, Lee J, Via LE, Lee S, Eum S, Lee S, Goldfeder LC, Cai Y, Jin B, Kim Y, Oh T, Chen RY, Dodd LE, Gu W, Dartois V, Park S, Kim CT, C. E. Cho S. Efficacy and safety of metronidazole for pulmonary multidrug-resistant tuberculosis. Antimicrob Agents Chemother. 2013; 57:3903–3909. [PubMed: 23733467]
66. Peirs P, Lefevre P, Boarbi S, Wang XM, Denis O, Braibant M, Pethe K, Locht C, Huygen K, Content J. Mycobacterium tuberculosis with disruption in genes encoding the phosphate binding

proteins PstS1 and PstS2 is deficient in phosphate uptake and demonstrates reduced in vivo virulence. Infection and Immunity. 2005; 73:1898–1902. [PubMed: 15731097]

67. Gouzy A, Poquet Y, Neyrolles O. Nitrogen metabolism in Mycobacterium tuberculosis physiology and virulence. Nat Rev Microbiol. 2014
68. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment. The Journal of experimental medicine. 2003; 198:693–704. [PubMed: 12953091]
69. Gomez JE, McKinney JD. M. tuberculosis persistence, latency, and drug tolerance. Tuberculosis (Edinb). 2004; 84:29–44. [PubMed: 14670344]
70. Pandey AK, ey, Sassetti CM. Mycobacterial persistence requires the utilization of host cholesterol. Proc Natl Acad Sci U S A. 2008; 105:4376–4380. [PubMed: 18334639]
71. Marrero J, Trujillo C, Rhee KY, Ehrt S. Glucose phosphorylation is required for Mycobacterium tuberculosis persistence in mice. PLoS Pathog. 2013; 9:e1003116. [PubMed: 23326232]
72. de Carvalho LP, Fischer SM, Marrero J, Nathan C, Ehrt S, Rhee KY. Metabolomics of Mycobacterium tuberculosis reveals compartmentalized co-catabolism of carbon substrates. Chemistry & Biology. 2010; 17:1122–1131. [PubMed: 21035735]
73. Silo-Suh L, Suh SJ, Phibbs PV, Ohman DE. Adaptations of Pseudomonas aeruginosa to the cystic fibrosis lung environment can include deregulation of zwf, encoding glucose-6-phosphate dehydrogenase. J Bacteriol. 2005; 187:7561–7568. [PubMed: 16267280]
74. Stanley SA, Grant SS, Kawate T, Iwase N, Shimizu M, Wivagg C, Silvis M, Kazyanskaya E, Aquadro J, Golas A, Fitzgerald M, Dai H, Zhang L, Hung DT. Identification of Novel Inhibitors of M. tuberculosis Growth Using Whole Cell Based High-Throughput Screening. 2012
75. Griffin JE, Pandey AK, Gilmore SA, Mizrahi V, McKinney JD, Bertozzi CR, Sassetti CM. Cholesterol catabolism by Mycobacterium tuberculosis requires transcriptional and metabolic adaptations. Chemistry & biology. 2012; 19:218–227. [PubMed: 22365605]
76. Thomas ST, VanderVen BC, Sherman DR, Russell DG, Sampson NS. Pathway profiling in Mycobacterium tuberculosis: elucidation of cholesterol-derived catabolite and enzymes that catalyze its metabolism. J Biol Chem. 2011; 286:43668–43678. [PubMed: 22045806]
77. Chang JC, Miner MD, Pandey AK, Gill WP, Harik NS, Sassetti CM, Sherman DR. igr Genes and Mycobacterium tuberculosis cholesterol metabolism. J Bacteriol. 2009; 191:5232–5239. [PubMed: 19542286]
78. Yam KC, D'Angelo I, Kalscheuer R, Zhu H, Wang JX, Snieckus V, Ly LH, Converse PJ, Jacobs WR Jr. Strynadka N, Eltis LD. Studies of a ring-cleaving dioxygenase illuminate the role of cholesterol metabolism in the pathogenesis of Mycobacterium tuberculosis. PLoS Pathog. 2009; 5:e1000344. [PubMed: 19300498]
79. Davis BD. Mechanism of bactericidal action of aminoglycosides. Microbiol Rev. 1987; 51:341–350. [PubMed: 3312985]
80. Chen CR, Malik M, Snyder M, Drlica K. DNA gyrase and topoisomerase IV on the bacterial chromosome: quinolone-induced DNA cleavage. Journal of Molecular Biology. 1996; 258:627–637. [PubMed: 8636997]
81. Savvi S, Warner DF, Kana BD, McKinney JD, Mizrahi V, Dawes SS. Functional characterization of a vitamin B12-dependent methylmalonyl pathway in Mycobacterium tuberculosis: implications for propionate metabolism during growth on fatty acids. J Bacteriol. 2008; 190:3886–3895. [PubMed: 18375549]
82. Lee W, VanderVen BC, Fahey RJ, Russell DG. Intracellular Mycobacterium tuberculosis Exploits Host-derived Fatty Acids to Limit Metabolic Stress. Journal of Biological Chemistry. 2013; 288:6788–6800. [PubMed: 23306194]
83. Gopinath K, Venclovas C, Ioerger TR, Sacchettini JC, McKinney JD, Mizrahi V, Warner DF. A vitamin B(1)(2) transporter in Mycobacterium tuberculosis. Open biology. 2013; 3:120175. [PubMed: 23407640]
84. Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol. 2013; 11:371–384. [PubMed: 23669886]

85. Potrykus J, Ballou ER, Childers DS, Brown AJ. Conflicting interests in the pathogen-host tug of war: fungal micronutrient scavenging versus mammalian nutritional immunity. PLoS Pathog. 2014; 10:e1003910. [PubMed: 24626223]

86. Wells RM, Jones CM, Xi Z, Speer A, Danilchanka O, Doornbos KS, Sun P, Wu F, Tian C, Niederweis M. Discovery of a siderophore export system essential for virulence of Mycobacterium tuberculosis. PLoS Pathog. 2013; 9:e1003120. [PubMed: 23431276]

87. Rodriguez GM, Smith I. Identification of an ABC transporter required for iron acquisition and virulence in Mycobacterium tuberculosis. J Bacteriol. 2006; 188:424–430. [PubMed: 16385031]

88. Owens CP, Chim N, Graves AB, Harmston CA, Iniguez A, Contreras H, Liptak MD, Goulding CW. The Mycobacterium tuberculosis secreted protein Rv0203 transfers heme to membrane proteins MmpL3 and MmpL11. J Biol Chem. 2013; 288:21714–21728. [PubMed: 23760277]

89. Botella H, Peyron P, Levillain F, Poincloux R, Poquet Y, Brandli I, Wang C, Tailleux L, Tilleul S, Charriere GM, Waddell SJ, Foti M, Lugo-Villarino G, Gao Q, Maridonneau-Parini I, Butcher PD, Castagnoli PR, Gicquel B, de Chastellier C, Neyrolles O. Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. Cell Host Microbe. 2011; 10:248–259. [PubMed: 21925112]

90. Ward SK, Abomoelak B, Hoye EA, Steinberg H, Talaat AM. CtpV: a putative copper exporter required for full virulence of Mycobacterium tuberculosis. Mol Microbiol. 2010; 77:1096–1110. [PubMed: 20624225]

91. Wagner D, Maser J, Moric I, Boechat N, Vogt S, Gicquel B, Lai B, Reyrat JM, Bermudez L. Changes of the phagosomal elemental concentrations by Mycobacterium tuberculosis Mramp. Microbiology. 2005; 151:323–332. [PubMed: 15632449]

92. Shi X, Festa RA, Ioerger TR, Butler-Wu S, Sacchettini JC, Darwin KH, Samanovic MI. The copper-responsive RicR regulon contributes to Mycobacterium tuberculosis virulence. mBio. 2014; 5

93. Padilla-Benavides T, Long JE, Raimunda D, Sassetti CM, Arguello JM. A novel P(1B)-type Mn2+-transporting ATPase is required for secreted protein metallation in mycobacteria. J Biol Chem. 2013; 288:11334–11347. [PubMed: 23482562]

94. Singh M, Jadaun GP, Ramdas, Srivastava K, Chauhan V, Mishra R, Gupta K, Nair S, Chauhan DS, Sharma VD, Venkatesan K, Katoch VM. Effect of efflux pump inhibitors on drug susceptibility of ofloxacin resistant Mycobacterium tuberculosis isolates. The Indian journal of medical research. 2011; 133:535–540. [PubMed: 21623040]

95. Martinez JL, Sanchez MB, Martinez-Solano L, Hernandez A, Garmendia L, Fajardo A, Alvarez-Ortega C. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS Microbiol Rev. 2009; 33:430–449. [PubMed: 19207745]

96. Adams KN, Takaki K, Connolly LE, Wiedenhoft H, Winglee K, Humbert O, Edelstein PH, Cosma CL, Ramakrishnan L. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell. 2011; 145:39–53. [PubMed: 21376383]

97. Talaat AM, Lyons R, Howard ST, Johnston SA. The temporal expression profile of Mycobacterium tuberculosis infection in mice. Proc Natl Acad Sci U S A. 2004; 101:4602–4607. [PubMed: 15070764]

98. Badarinarayana V, Estep PW 3rd, Shendure J, Edwards J, Tavazoie S, Lam F, Church GM. Selection analyses of insertional mutants using subgenic-resolution arrays. Nat Biotechnol. 2001; 19:1060–1065. [PubMed: 11689852]

99. Wixon J. Featured organism: reductive evolution in bacteria: Buchnera sp., Rickettsia prowazekii and Mycobacterium leprae. Comp Funct Genomics. 2001; 2:44–48. [PubMed: 18628941]

100. Tan S, Sukumar N, Abramovitch RB, Parish T, Russell DG. Mycobacterium tuberculosis responds to chloride and pH as synergistic cues to the immune status of its host cell. PLoS Pathog. 2013; 9:e1003282. [PubMed: 23592993]

101. Potzkei J, Kunze M, Drepper T, Gensch T, Jaeger KE, Buchs J. Real-time determination of intracellular oxygen in bacteria using a genetically encoded FRET-based biosensor. BMC Biol. 2012; 10:28. [PubMed: 22439625]

102. Berg J, Hung YP, Yellen G. A genetically encoded fluorescent reporter of ATP:ADP ratio. Nature methods. 2009; 6:161–166. [PubMed: 19122669]

103. Gabriel GV, Viviani VR. Novel application of pH-sensitive firefly luciferases as dual reporter genes for simultaneous ratiometric analysis of intracellular pH and gene expression/location. Photochem Photobiol Sci. 2014

104. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nature reviews. Drug discovery. 2007; 6:29–40.

105. Chan PF, Holmes DJ, Payne DJ. Finding the gems using genomic discovery: antibacterial drug discovery strategies – the successes and the challenges. Drug Discovery Today: Therapeutic Strategies. 2004; 1:519–527.

106. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. Nature. 2011; 469:483–490. [PubMed: 21270886]

107. Lechartier B, Rybniker J, Zumla A, Cole ST. Tuberculosis drug discovery in the post-post-genomic era. EMBO Mol Med. 2014 n/a-n/a.

108. Gould TA, Langemheen H, Muñoz-Elías EJ, McKinney JD, Sacchettini JC. Dual role of isocitrate lyase 1 in the glyoxylate and methylcitrate cycles in Mycobacterium tuberculosis. Mol Microbiol. 2006; 61:940–947. [PubMed: 16879647]

109. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci U S A. 1997; 94:5243–5248. [PubMed: 9144222]

110. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science (New York, N.Y.). 2003; 302:654–659.

111. Vandal OH, Pierini LM, Schnappinger D, Nathan CF, Ehrt S. A membrane protein preserves intrabacterial pH in intraphagosomal Mycobacterium tuberculosis. Nature Medicine. 2008; 14:849–854.

112. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment. The Journal of experimental medicine. 2003; 198:693–704. [PubMed: 12953091]

113. Marrero J, Rhee KY, Schnappinger D, Pethe K, Ehrt S. Gluconeogenic carbon flow of tricarboxylic acid cycle intermediates is critical for Mycobacterium tuberculosis to establish and maintain infection. Proc Natl Acad Sci U S A. 2010; 107:9819–9824. [PubMed: 20439709]

114. Gold, Deng H, Bryk R, Vargas D, Eliezer D, Roberts J, Jiang X, Nathan C. Identification of a copper-binding metallothionein in pathogenic mycobacteria. Nature chemical biology. 2008; 4:609–616.

115. Gold B, Rodriguez GM, Marras SA, Pentecost M, Smith I. The Mycobacterium tuberculosis IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. Mol Microbiol. 2001; 42:851–865. [PubMed: 11722747]

116. Timm J, Post FA, Bekker L, Walther GB, Wainwright HC, Manganelli R, Chan W, Tsenova L, Gold B, Smith I, Kaplan G, McKinney JD. Differential expression of iron-, carbon-, and oxygen-responsive mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients. Proc Natl Acad Sci U S A. 2003; 100:14321–14326. [PubMed: 14623960]

117. Walters SB, Dubnau E, Kolesnikova I, Laval F, Daffe M, Smith I. The Mycobacterium tuberculosis PhoPR two-component system regulates genes essential for virulence and complex lipid biosynthesis. Mol Microbiol. 2006; 60:312–330. [PubMed: 16573683]

118. Miner MD, Chang JC, P AK, Pandey AK, ey, Sassetti CM, Sherman DR. Role of cholesterol in Mycobacterium tuberculosis infection. Indian journal of experimental biology. 2009; 47:407–411. [PubMed: 19634704]

119. Gold, Pingle M, Brickner SJ, Shah N, Roberts J, Rundell M, Bracken WC, Warrier T, Somersan S, Venugopal A, Darby C, Jiang X, Warren JD, Fernandez J, Ouerfelli O, Nuermberger EL, Cunningham-Bussel A, Rath P, Chidawanyika T, Deng H, Realubit R, Glickman JF, Nathan CF. Nonsteroidal anti-inflammatory drug sensitizes Mycobacterium tuberculosis to endogenous and exogenous antimicrobials. Proc Natl Acad Sci U S A. 2012; 109:16004–16011. [PubMed: 23012453]

120. Khare G, Kumar P, Tyagi AK. Whole-cell screening-based identification of inhibitors against the intraphagosomal survival of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2013; 57:6372–6377. [PubMed: 24060878]

121. Wei J, Krishnamoorthy V, Murphy K. Depletion of antibiotic targets has widely varying effects on growth. 2011 in Proceedings of the.

122. Tobin DM, Vary JC Jr. Ray JP, Walsh GS, Dunstan SJ, Bang ND, Hagge DA, Khadge S, King MC, Hawn TR, Moens CB, Ramakrishnan L. The lta4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans. Cell. 2010; 140:717–730. [PubMed: 20211140]

123. Abrahams GL, Kumar A, Savvi S, Hung AW, Wen S, Abell C, C. E. Sherman DR, Boshoff HI, Mizrahi V. Pathway-selective sensitization of Mycobacterium tuberculosis for target-based whole-cell screening. Chemistry & Biology. 2012; 19:844–854. [PubMed: 22840772]

124. DeVito JA, Mills JA, Liu VG, Agarwal A, Sizemore CF, Yao Z, Stoughton DM, Cappiello MG, Barbosa MD, Foster LA, Pompliano DL. An array of target-specific screening strains for antibacterial discovery. Nat Biotechnol. 2002; 20:478–483. [PubMed: 11981561]

125. Forsyth RA, Haselbeck RJ, Ohlsen KL, Yamamoto RT, Xu H, Trawick JD, Wall D, Wang L, Brown-Driver V, Froelich JM, C KG, King P, McCarthy M, Malone C, Misiner B, Robbins D, Tan Z, Zhu Zy ZY, Carr G, Mosca DA, Zamudio C, Foulkes JG, Zyskind JW. A genome-wide strategy for the identification of essential genes in Staphylococcus aureus. Mol Microbiol. 2002; 43:1387–1400. [PubMed: 11952893]

126. Young K, Jayasuriya H, Ondeyka JG, Herath K, Zhang C, Kodali S, Galgoci A, Painter R, Brown-Driver V, Yamamoto R, Silver LL, Zheng Y, Ventura JI, Sigmund J, Ha S, Basilio A, Vicente F, Tormo JR, Pelaez F, Youngman P, Cully D, Barrett JF, Schmatz D, Singh SB, Wang J. Discovery of FabH/FabF inhibitors from natural products. Antimicrob Agents Chemother. 2006; 50:519–526. [PubMed: 16436705]

127. Kim J, O'Brien KM, Sharma R, Boshoff HI, Rehren G, Chakraborty S, Wallach JB, Monteleone M, Wilson DJ, Aldrich CC, C. E. Rhee KY, Ehrt S, Schnappinger D. A genetic strategy to identify targets for the development of drugs that prevent bacterial persistence. Proc Natl Acad Sci U S A. 2013; 110:19095–19100. [PubMed: 24191058]

128. Roemer T, Krysan DJ. Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harb Perspect Med. 2014; 4

129. Klotzsche M, Ehrt S, Schnappinger D. Improved tetracycline repressors for gene silencing in mycobacteria. Nucleic Acids Res. 2009; 37:1778–1788. [PubMed: 19174563]

130. Ehrt S, Guo XV, Hickey CM, Ryou M, Monteleone M, Riley LW, Schnappinger D. Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. Nucleic Acids Res. 2005; 33:e21. [PubMed: 15687379]

131. Darby CM, Ingólfsson HI, Jiang X, Shen C, Sun M, Zhao N, Burns K, Liu G, Ehrt S, Warren JD, Anderson OS, Brickner SJ, Nathan C. Whole cell screen for inhibitors of pH homeostasis in Mycobacterium tuberculosis. PLoS One. 2013; 8:e68942. [PubMed: 23935911]

132. Tong AH, Lesage G, Bader GD, Ding H, Xu H, Xin X, Young J, Berriz GF, Brost RL, Chang M, Chen Y, Cheng X, Chua G, Friesen H, Goldberg DS, Haynes J, Humphries C, He G, Hussein S, Ke L, Krogan N, Li Z, Levinson JN, Lu H, Menard P, Munyana C, Parsons AB, Ryan O, Tonikian R, Roberts T, Sdicu AM, Shapiro J, Sheikh B, Suter B, Wong SL, Zhang LV, Zhu H, Burd CG, Munro S, Sander C, Rine J, Greenblatt J, Peter M, Bretscher A, Bell G, Roth FP, Brown GW, Andrews B, Bussey H, Boone C. Global mapping of the yeast genetic interaction network. Science. 2004; 303:808–813. [PubMed: 14764870]

133. Forgacs P, Wengenack NL, Hall L, Zimmerman SK, Silverman ML, Roberts GD. Tuberculosis and trimethoprim-sulfamethoxazole. Antimicrob Agents Chemother. 2009; 53:4789–4793. [PubMed: 19564358]

134. Vilchéze C, Jacobs WR. The combination of sulfamethoxazole, trimethoprim, and isoniazid or rifampin is bactericidal and prevents the emergence of drug resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2012; 56:5142–5148. [PubMed: 22825115]

135. Lorenzo S, Alffenaar JW, Sotgiu G, Centis R, D'Ambrosio L, Tiberi S, Bolhuis MS, Altena R, Viggiani P, Piana A, Spanevello A, Migliori GB. Efficacy and safety of meropenem-clavulanate added to linezolid-containing regimens in the treatment of MDR-/XDR-TB. The European respiratory journal. 2013; 41:1386–1392. [PubMed: 22997218]

136. Ch'ng J, Mok S, Bozdech Z, Lear MJ, Boudhar A, Russell B, Nosten F, Tan KS. A whole cell pathway screen reveals seven novel chemosensitizers to combat chloroquine resistant malaria. Scientific reports. 2013; 3:1734. [PubMed: 23615863]

137. Tan CM, Therien AG, Lu J, Lee SH, Caron A, Gill CJ, Lebeau-Jacob C, Benton-Perdomo L, Monteiro JM, Pereira PM, Elsen NL, Wu J, Deschamps K, Petcu M, Wong S, Daigneault E, Kramer S, Liang L, Maxwell E, Claveau D, Vaillancourt J, Skorey K, Tam J, Wang H, Meredith TC, Sillaots S, Wang-Jarantow L, Ramtohul Y, Langlois E, Landry F, Reid JC, Parthasarathy G, Sharma S, Baryshnikova A, Lumb KJ, Pinho MG, Soisson SM, Roemer T. Restoring methicillin-resistant Staphylococcus aureus susceptibility to β-lactam antibiotics. Sci Transl Med. 2012; 4:126ra135.

138. Lee SH, Jarantow LW, Wang H, Sillaots S, Cheng H, Meredith TC, Thompson J, Roemer T. Antagonism of chemical genetic interaction networks resensitize MRSA to β-lactam antibiotics. Chemistry & Biology. 2011; 18:1379–1389. [PubMed: 22118672]

139. Jindani A, Dore CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. American journal of respiratory and critical care medicine. 2003; 167:1348–1354. [PubMed: 12519740]

140. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2000; 4:796–806.

141. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, Dowd CS, Lee IY, Kim P, Zhang L, Kang S, Keller TH, Jiricek J, C. E. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. Science (New York, N.Y.). 2008; 322:1392–1395.

142. Nuermberger E, Tyagi S, Tasneen R, Williams KN, Almeida D, Rosenthal I, Grosset JH. Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin, and pyrazinamide in a murine model of tuberculosis. Antimicrob Agents Chemother. 2008; 52:1522–1524. [PubMed: 18285479]

143. Diacon AH, Dawson R, Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. Lancet. 2012; 380:986–993. [PubMed: 22828481]

144. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002; 347:975–982. [PubMed: 12324553]

145. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, Derrick SC, Shi R, Kumar NP, Wei W, Yuan X, Zhang G, Cai Y, Babu S, Catalfamo M, Salazar AM, Via LE, Barry CE 3rd, Sher A. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature. 2014; 511:99–103. [PubMed: 24990750]

146. Stanley SA, Barczak AK, Silvis MR, Luo SS, Sogi K, Vokes M, Bray M, Carpenter AE, Moore CB, Siddiqi N, Rubin EJ, Hung DT. Identification of host-targeted small molecules that restrict intracellular Mycobacterium tuberculosis growth. PLoS Pathog. 2014; 10:e1003946. [PubMed: 24586159]

147. Wu K, Koo J, Jiang X, Chen R, Cohen SN, Nathan C. Improved control of tuberculosis and activation of macrophages in mice lacking protein kinase R. PLoS One. 2012; 7:e30512. [PubMed: 22359543]

148. Tobin D, Roca F, Oh S, McFarl R, Vickery T, Ray J, Ko D, Zou Y, Bang N, Chau TH, Vary J, Hawn T, Dunstan S, Farrar J, Thwaites G, King M, Serhan C, Ramakrishnan L, McFarland R. Host Genotype-Specific Therapies Can Optimize the Inflammatory Response to Mycobacterial Infections. Cell. 2012; 148:434–446. [PubMed: 22304914]

149. Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. Nature Reviews Drug Discovery. 2012; 11:709–730.

150. Gumbo T, Louie A, Deziel MR, Drusano GL. Pharmacodynamic evidence that ciprofloxacin failure against tuberculosis is not due to poor microbial kill but to rapid emergence of resistance. Antimicrob Agents Chemother. 2005; 49:3178–3181. [PubMed: 16048921]

151. Kjellsson MC, Via LE, Goh A, Weiner D, Low KM, Kern S, Pillai G, C. E. Dartois V. Pharmacokinetic evaluation of the penetration of antituberculosis agents in rabbit pulmonary lesions. Antimicrob Agents Chemother. 2012; 56:446–457. [PubMed: 21986820]

152. Prideaux B, Dartois V, Staab D, Weiner DM, Goh A, Via LE, C. E. Stoeckli M. High-sensitivity MALDI-MRM-MS imaging of moxifloxacin distribution in tuberculosis-infected rabbit lungs and granulomatous lesions. Anal Chem. 2011; 83:2112–2118. [PubMed: 21332183]

153. Lenaerts AJ, Gruppo V, Brooks JV, Orme IM. Rapid in vivo screening of experimental drugs for tuberculosis using gamma interferon gene-disrupted mice. Antimicrob Agents Chemother. 2003; 47:783–785. [PubMed: 12543692]

154. Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. Infection and Immunity. 1999; 67:4531–4538. [PubMed: 10456896]

155. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet. 2001; 358:1687–1693. [PubMed: 11728545]

156. Davies SJ, Lim KC, Blank RB, Kim JH, Lucas KD, Hernandez DC, Sedgwick JD, McKerrow JH. Involvement of TNF in limiting liver pathology and promoting parasite survival during schistosome infection. Int J Parasitol. 2004; 34:27–36. [PubMed: 14711587]

157. Lin PL, Plessner HL, Voitenok NN, Flynn JL. Tumor necrosis factor and tuberculosis. J Investig Dermatol Symp Proc. 2007; 12:22–25.

158. Subbian S, Tsenova L, O'Brien P, Yang G, Koo MS, Peixoto B, Fallows D, Dartois V, Muller G, Kaplan G. Phosphodiesterase-4 inhibition alters gene expression and improves isoniazid-mediated clearance of Mycobacterium tuberculosis in rabbit lungs. PLoS Pathog. 2011; 7:e1002262. [PubMed: 21949656]

159. Skerry C, Harper J, Klunk M, Bishai WR, Jain SK. Adjunctive TNF inhibition with standard treatment enhances bacterial clearance in a murine model of necrotic TB granulomas. PLoS One. 2012; 7:e39680. [PubMed: 22761866]

160. Wallis RS, Kyambadde P, Johnson JL, Horter L, Kittle R, Pohle M, Ducar C, Millard M, Mayanja-Kizza H, Whalen C, Okwera A. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. Aids. 2004; 18:257–264. [PubMed: 15075543]

161. Dutta NK, Mazumdar K, Dastidar SG, Park JH. Activity of diclofenac used alone and in combination with streptomycin against Mycobacterium tuberculosis in mice. Int J Antimicrob Agents. 2007; 30:336–340. [PubMed: 17644321]

162. Byrne ST, Denkin SM, Zhang Y. Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. J Antimicrob Chemother. 2007; 59:313–316. [PubMed: 17185297]

163. Vilaplana C, Marzo E, Tapia G, Diaz J, Garcia V, Cardona PJ. Ibuprofen therapy resulted in significantly decreased tissue bacillary loads and increased survival in a new murine experimental model of active tuberculosis. J Infect Dis. 2013; 208:199–202. [PubMed: 23564636]

164. Hnizdo E, Singh T, Churchyard G. Chronic pulmonary function impairment caused by initial and recurrent pulmonary tuberculosis following treatment. Thorax. 2000; 55:32–38. [PubMed: 10607799]

165. Zhang G, Zhou B, Li S, Yue J, Yang H, Wen Y, Zhan S, Wang W, Liao M, Zhang M, Zeng G, Feng CG, Sassetti CM, Chen X. Allele-Specific Induction of IL-1beta Expression by C/EBPbeta and PU.1 Contributes to Increased Tuberculosis Susceptibility. PLoS Pathog. 2014; 10:e1004426. [PubMed: 25329476]

166. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, Lopez CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2013; 110:3507–3512. [PubMed: 23401516]

167. Dutta NK, Mehra S, Didier PJ, Roy CJ, Doyle LA, Alvarez X, Ratterree M, Be NA, Lamichhane G, Jain SK, Lacey MR, Lackner AA, Kaushal D. Genetic requirements for the survival of tubercle bacilli in primates. J Infect Dis. 2010; 201:1743–1752. [PubMed: 20394526]

168. Ahmad Z, Fraig MM, Pinn ML, Tyagi S, Nuermberger EL, Grosset JH, Karakousis PC. Effectiveness of tuberculosis chemotherapy correlates with resistance to Mycobacterium tuberculosis infection in animal models. J Antimicrob Chemother. 2011; 66:1560–1566. [PubMed: 21602551]

169. Vandal OH, Nathan CF, Ehrt S. Acid resistance in Mycobacterium tuberculosis. J Bacteriol. 2009; 191:4714–4721. [PubMed: 19465648]

170. Schaible UE, Kaufmann SH. Iron and microbial infection. Nat Rev Microbiol. 2004; 2:946–953. [PubMed: 15550940]

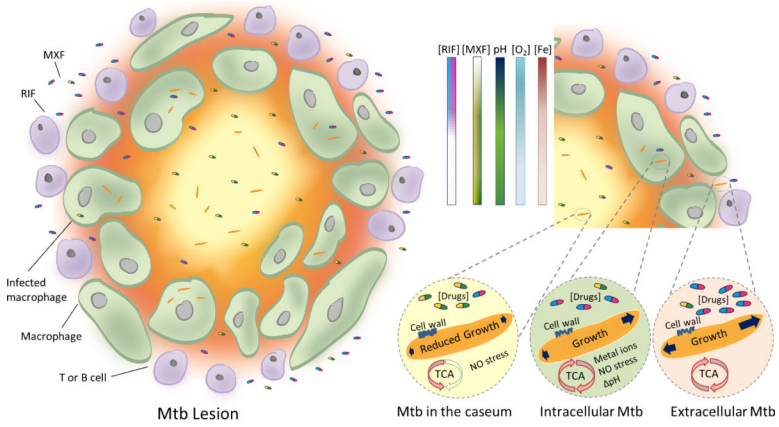

Fig. 1. Macrophages, B cells, and T cells surround a necrotic, caseous center in Mtb lesions

Mtb experiences a wide variety of changing stresses in the various lesion microenvironments, including altered pH (169), oxygen tension (26) and iron availability (170). In addition, the lesional penetration of standard first-line antibiotics varies greatly, exposing the bacteria to range of drug concentrations (4). Resulting changes in bacterial physiology lead to a mixed mycobacteria population in a variety of metabolic states, complicating treatment.

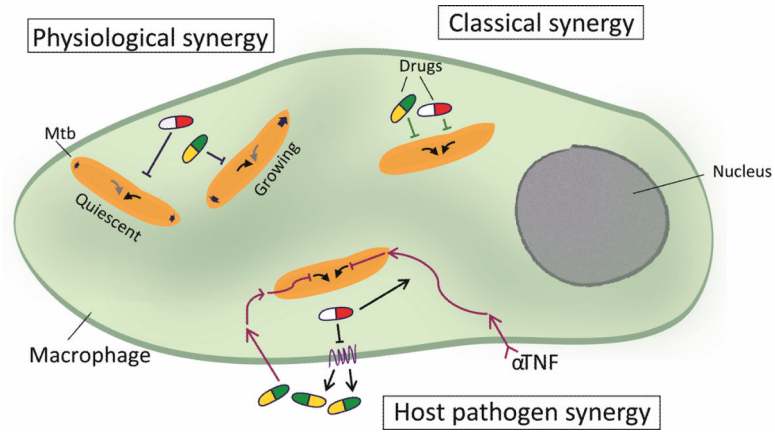

Fig. 2. The three types of synergy offer opportunities for novel approaches to anti-mycobacterial therapies

Classical synergy between two compounds occurs when multiple pathways are targeted simultaneously. Physiological synergy involves combination therapy with compounds that target different metabolic states. Host-pathogen synergy involves altering bacterial exposure to antibiotics by inhibiting host pathways that limit localized concentration, such as efflux pumps.