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MINIREVIEW

The current state of animal models and genomic approaches towards identifying and validating molecular determinants of *Mycobacterium tuberculosis* infection and tuberculosis disease

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One sentence summary: This review discusses the current state of the important animal models and techniques being used to study *Mycobacterium tuberculosis* infection and tuberculosis disease as it occurs in humans.

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ABSTRACT

Animal models are important in understanding both the pathogenesis of and immunity to tuberculosis (TB). Unfortunately, we are beginning to understand that no animal model perfectly recapitulates the human TB syndrome, which encompasses numerous different stages. Furthermore, *Mycobacterium tuberculosis* infection is a very heterogeneous event at both the levels of pathogenesis and immunity. This review seeks to establish the current understanding of TB pathogenesis and immunity, as validated in the animal models of TB in active use today. We especially focus on the use of modern genomic approaches in these models to determine the mechanism and the role of specific molecular pathways. Animal models have significantly enhanced our understanding of TB. Incorporation of contemporary technologies such as single cell transcriptomics, high-parameter flow cytometric immune profiling, proteomics, proteomic flow cytometry and immunocytometry into the animal models in use will further enhance our understanding of TB and facilitate the development of treatment and vaccination strategies.

Keywords: *Mycobacterium tuberculosis*; animal models; translation

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INTRODUCTION

Tuberculosis (TB) is a widespread infectious disease, latently infecting up to one third of the world's population and causing ~1.3 million deaths in 2016 (WHO 2017). Many of the world's regions with high disease prevalence are rife with malnutrition, have limited access to medical resources and are hotbeds for non-compliance with medical interventions, leading to multidrug resistant and extensively drug-resistant strains (Gandhi et al. 2006; Migliori et al. 2012).

Mycobacterium tuberculosis (*Mtb*) is a pathogen that is spread by aerosol droplets to uninfected individuals when infected patients cough (Figure 1). In the lungs, *Mtb* comes into contact with innate immune cells that attempt to eliminate the pathogen. When *Mtb* comes into contact with alveolar macrophages, it can infect the cells and persist following phagocytosis as an intracellular pathogen. During the innate immune phase, inflammatory cells, including dendritic cells, neutrophils and macrophages, traffic to the lungs and attempt to control the infection (Nunes-Alves et al. 2014). Dissemination of *Mtb* to the lymph nodes occurs, allowing dendritic cells to present bacterial antigens to T cells and prime them (Chackerian et al. 2002; Wolf et al. 2008). Priming of ESAT6 in particular occurs ~10 days post-infection in the mediastinal lymph nodes and is followed later by generation of effector T cells (Reiley et al. 2008). The adaptive immune response that follows T cell priming, particularly the generation of effector T cells and Th1 CD4⁺ T cells, leads to the formation of granulomas. Granulomas are organized structures where T cells and B cells encapsulate innate immune cells, such as activated neutrophils and macrophages, with a fibrotic capsule to create a gradient of hypoxia, with the greatest concentration of hypoxia formed in its center to isolate and prevent the growth of *Mtb* (Kumar et al. 2011). Granulomas caseate and fail to contain *Mtb* when host-specific responses are insufficient to prevent bacterial replication, *Mtb*-infected macrophages necrotize and bacilli escape the hypoxic core to disseminate into uninfected lung tissues or secondary sites of infection including the liver, spleen and brain. *Mtb* has evolved numerous mechanisms of host resistance including its ability to alter T cell cytokines in infected cells, such as interleukin-1 β produced by dendritic cells, to induce Th2 immune responses and inhibit protective Th1 host responses (Dwivedi et al. 2012).

When patients are infected with *Mtb*, there are multiple clinical outcomes that they can experience. Many patients will control the infection and are considered latently infected (LTBI). These individuals typically possess just a few intact granulomas and are positive on the Mantoux tuberculin skin test (TST). About 10% of all patients with TB will progress to active infection over the course of their lifetimes, some with early progression (Kwan and Ernst 2011). Comorbidities can also induce progression to active disease in latently infected patients, termed reactivation, including, but not limited to, type 2 diabetes (T2D) and human immunodeficiency virus (HIV) (Kwan and Ernst 2011; Ai et al. 2016). HIV alone increases the rate of activation from 10% over a patient's lifetime to 10% per year (Kwan and Ernst 2011). Due to the wide variety of potential states and outcomes, we currently lack the knowledge and tools to distinguish which individuals have cleared or will control *Mtb* and which will progress to active TB disease. The lack of information on responses that are associated with subsequent progression to active TB impedes identification of individuals at the highest risk of progression to active TB and who would benefit most from preventative therapy in resource-poor, high TB-prevalent regions. Additionally, the lack of information on favorable immune

responses that lead to containment of infection, as well as their distinctions from those associated with progression to active disease, is a significant impediment to the design and evaluation of efficacious TB vaccines. Although it is generally understood that Th1 responses, such as IFN- γ that are generated in response to mycobacterial antigens, are protective, Th1 responses during active disease are unable to sterilize the host, complicating our ability to measure successful vaccination (Nunes-Alves et al. 2014).

As many mechanisms of pathogenesis and host response to infection are poorly understood, it is imperative to utilize animal models in order to investigate them. Animal models of TB infection and TB/HIV co-infection have the potential to yield insights about the host's responses to different strains, to different therapeutics and vaccines. They also have the ability to faithfully replicate comorbidities, particularly in the use of non-human primates (NHPs) with TB that are co-infected with simian immunodeficiency virus (SIV). The model used for studies, however, should always be carefully considered to maximize homology to human disease or symptomatology. For example, zebrafish infected with *M. marinum* have been a vital host to study caseous pathology within granulomas (Swaim et al. 2006; Cronan and Tobin 2014) despite being a non-mammalian organism. Balancing the ethical costs of the model, as well as the knowledge gap under investigation, are essential when choosing the proper animal model for studying TB infection. Here we discuss the most prominently used animal models of *Mtb* infection and their specific role in the identification of molecular determinants of TB pathology and immunity. We also describe the use of the various contemporary genome-wide approaches in the animal models of TB.

ANIMAL MODELS OF TB

Numerous animal models have been utilized for mechanistic and vaccine studies for TB. These range from more accurate, but more expensive, NHPs, to small and non-mammalian organisms like zebrafish. While NHPs best recapitulate the human immune response and susceptibility to TB, often smaller animal models like mice, rats, guinea pigs, rabbits and zebrafish are better utilized for investigating more narrow aspects of the immune response to mycobacteria, such as granuloma formation, susceptibility to different strains, or pre-clinical vaccine responses. A summary of the characteristics of each model is shown in Table 1.

NHPS

NHPs have been actively used primarily in the national primate research centers. The seven national primate research centers possess specialized personnel, large facilities and a wealth of expertise and experience that allows complex NHP research to be conducted safely and effectively in a BSL3 setting. In addition, several other research centers and universities in the USA and around the world have developed facilities to leverage NHP models in addition to smaller animal models such as mice, rabbits, guinea pigs and rats. The use of NHPs, particularly macaques, as models of human disease has significantly increased recently. Of the >15 000 publications in PubMed that utilized macaque models of human disease, 60% were published in the last 10 years. These systems have also been significantly utilized in the modeling of infectious diseases, particularly HIV (~4500 publications) (Hulskotte, Geretti and Osterhaus 1998; Veazey et al.

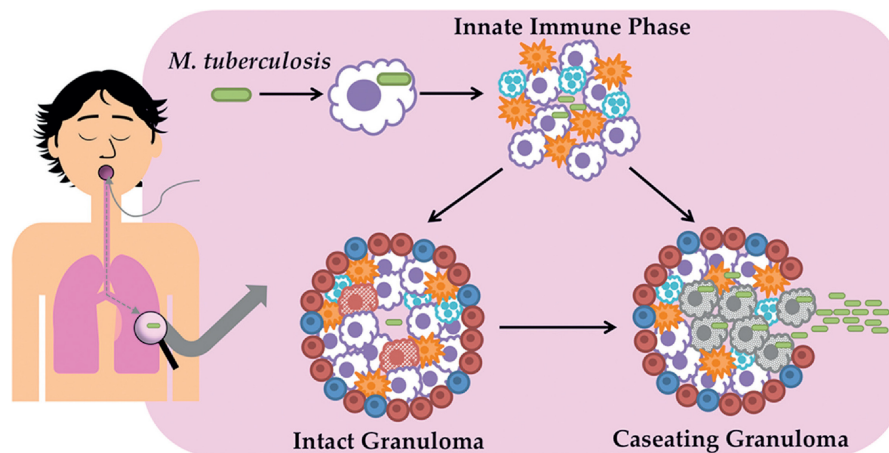


Figure 1 *Mycobacterium tuberculosis* (*Mtb*) is spread via aerosols and is harbored primarily in the lungs. When macrophages encounter and engulf the pathogens, it may be eliminated or may persist due to compensatory mechanisms. During the innate immune phase, macrophages, neutrophils and dendritic cells are recruited to the site of infection. After several weeks, T and B cells migrate to the site of infection and form granulomas that encapsulate the innate immune cells and bacilli in a fibrotic capsule that creates a gradient of hypoxia. If the immune cells are not able to kill the bacilli and prevent its growth, granulomas caseate and *Mtb* bacilli disseminate to other organs including the spleen, liver and brain.

Table 1. Comparison of biosafety safety level 2 (BSL-2) and BSL-3 animal models of *Mtb* infection.

	Non-human primates	Mouse	Rabbit	Guinea pig	Rat	Zebrafish
Susceptible to human clinical strains of <i>Mtb</i>	Yes	Yes	Yes	Yes	Yes	No
Potential for PK studies	Yes, but significant cost	No, differs from humans	Yes	No, differs from humans	Yes	No
BSL-2 model	No	No	No	No	No	Yes
BSL-3 model	Yes	Yes	Yes	Yes	Yes	No
Easy to house	No	Yes	No	No	Yes	Yes
Pulmonary pathology	Yes	Yes	Yes	Yes	Yes	No
Caseous pathology	Yes	No, except C3HeB/Fej mouse	Yes	Yes	Yes	Yes
Cavitary lesions	Yes	No	Yes	No	No	Yes
Dissemination	Yes	Yes	Yes	Yes	Yes	Yes
Latency	Yes	No	Yes	No	Yes	Yes
Ready availability of immunological reagents	Yes, human cross-reactivity	Yes	No	No	No	No
CD1-cross reactivity	CD1a, CD1b, CD1c, CD1d	CD1d only	CD1a, CD1b, CD1d	CD1b, CD1c	CD1d only	No

1998; Douek et al. 2002; Franchini et al. 2002; Brechley et al. 2004; Hatzioannou et al. 2009; Weed et al. 2012; Kimata 2014), HSV (Peretti et al. 2005; Crostarosa et al. 2009; Calenda et al. 2017), influenza (Brown et al. 2010; Kitano et al. 2010; McDermott et al. 2011), *Streptococcus pneumoniae* (Philipp et al. 2012), *Neisseria gonorrhoeae* (Weyand et al. 2013), non-tuberculous mycobacteria (Winthrop et al. 2010; Henkle and Winthrop 2015) and malaria (Lombardini et al. 2015; Phares et al. 2017). These models have not only investigated the pathogenesis of infection, but have also related tissue pathology, vaccine and drug development and, of late, host-directed or immunotherapeutics (Kaushal et al. 2015).

Macaques have been extensively used in TB research (~150 publications) (Kaushal and Mehra 2012; Kaushal et al. 2012; Foreman et al. 2017). In fact, macaques were extensively used for studying experimental *Mtb* infections in the early and middle parts of the 20th century, but as the advent of antibiotics and the widespread use of the Bacillus Calmette-Guérin (BCG) vaccine resulted in reduced TB rates, interest waned. Barclay led key

studies five decades ago where Indian rhesus macaques were infected via the aerosol route with *Mtb*. (Barclay et al. 1970). Barclay et al. tested both BCG and *Mtb* cell wall as effective vaccines against *Mtb* challenge in this species (Ribi et al. 1971; Anacker et al. 1972), even demonstrating the feasibility of utilizing aerosol BCG vaccination to protect against TB challenge (Barclay et al. 1973). As TB disease resurged in the developed world in the 1980s and 1990s, this led to renewed interest in its research and it was at once clear that the model systems available to study TB at that time were insufficient. Walsh led the study of *Mtb* infection in the modern era in cynomolgus macaques and showed that it was possible to generate human-like LTBI in that species (Walsh et al. 1996). The cynomolgus macaque was then extensively used, primarily by Flynn and colleagues, to study aspects of *Mtb* infection (Capuano et al. 2003; Lin et al. 2009), especially early infection (Lin et al. 2006), reactivation due to SIV co-infection (Diedrich et al. 2010) and for vaccine evaluation (Lin et al. 2012; Mehra et al. 2013). The work of several other groups

simultaneously led to a renewed interest in the rhesus macaque as a model for TB pathogenesis (Gormus et al. 2004; Lewinsohn et al. 2006; Qiu et al. 2008; Chen et al. 2009; Sharpe et al. 2009; Verreck et al. 2009; Sharpe et al. 2010). *Mtb*-infected rhesus macaques have since been utilized extensively to study the role of bacterial determinants of pathogenesis (Dutta et al. 2010), e.g. stress-response transcription factors (Mehra et al. 2012, 2015). These studies generated results different to those obtained in the typical mouse model and extended our knowledge of how *Mtb* has evolved to manage stress in its attempt to persist. This model has been invaluable to replicate the immune responses that lead to human granuloma formation and their failure because NHPs are able to produce granulomas that are comparable with those found in humans (Figure 1) (Mehra et al. 2010, 2013; Slight et al. 2013). The NHP model has been vital for testing vaccines and vaccine-induced responses (Kaushal et al. 2015; Foreman et al. 2017), in elucidating *Mtb*/HIV co-infections using SIV as a surrogate (Diedrich et al. 2010; Sestak et al. 2011; Foreman et al. 2016) and in ascertaining the role of individual cell types (Kuroda et al. 2018) and immune/metabolic pathways/molecules (Diedrich, Mattila and Flynn 2013; Cheng et al. 2017).

Old World NHPs originating from Africa and Asia, including macaques, are prevalent as an NHP model in TB research. New World NHPs, originating from Central and South America as well as Mexico, e.g. marmosets, have also been used for TB research. A challenge of the marmoset model remains that even low-dose infection has been shown to yield intensely pathological outcomes, making it difficult to faithfully model TB latency (Via et al. 2013; Cadena et al. 2016). Marmosets remain a captivating model organism, however, due to the ability to standardize experiments using twins, particularly for comparisons of strain virulence and disease progression (Via et al. 2013). Marmosets have also been a useful model for investigating the effect of different drug regimens because they produce fully formed granulomas that can be collected and evaluated to determine if a drug has fully sterilized a granuloma (Via et al. 2015). This is a method that has been evaluated additionally in cynomolgus macaques (Gideon et al. 2015). There is limited work on TB in baboons, although that species of monkeys is also susceptible to *Mtb* under experimental conditions (Martino, Hubbard and Schlabritz-Loutsevitch 2007). It may be useful to look at the effectiveness of the baboon as a model of TB co-infection because extensive work has been done in baboons to study T2D (Rincon-Choles et al. 2012). T2D is known to promote *Mtb* infection and TB disease (Gauld and Lyall 1947; Kapur and Harries 2013). Treatment strategies for T2D have exhibited promise as anti-TB host-directed therapeutics (Singhal et al. 2014).

In terms of determining which species to use as a model for TB, an understanding of what one wishes to accomplish must be reached, considering both the strain of *Mtb* being used as well as what effect is being investigated. In side-by-side comparisons of rhesus macaques and cynomolgus macaques, the former have been found to be more susceptible to developing active TB infections when infected with a virulent strain, *Mtb* Erdman (Langermans et al. 2001; Sharpe et al. 2009; Maiello et al. 2017). The lowest dose used of *Mtb* Erdman by Sharpe et al., however, was ~30 CFU, with an upper limit of 500 CFU (Sharpe et al. 2009). In a later study, where a retained aerosol exposure was calculated to be as low as 3 CFU of *Mtb* Erdman, Sharpe et al. still found that rhesus macaques were overall more likely to progress to active disease and that cynomolgus macaques possessed a reduced disease burden (Sharpe et al. 2016). When a less virulent strain is used, such as CDC1551, it is possible to investigate latency even in rhesus macaques (Sestak et al. 2011; Foreman et al. 2016).

VALIDATION OF MOLECULAR DETERMINANTS OF INFECTION, DISEASE AND PROTECTION IN THE NHP MODEL

Over the last decade, important discoveries have been made using innovative approaches, including novel genomic techniques, to validate molecular determinants of infection and disease in NHPs. In 2007, Huang et al. established and validated a real-time quantitation system that identified 78 immune genes that were induced in rhesus macaques that were vaccinated/challenged with BCG (Huang et al. 2007). The first application of a system-wide approach in the NHP model of TB infection showed that gene expression in recently formed lung granulomas is characterized by a massive cytokine storm (Mehra et al. 2010). However, with the establishment of the chronic state of infection, this intense expression of pro-inflammatory cytokines was rapidly inhibited in later granulomas (Mehra et al. 2010). This study provided significant insights into the functioning of the primate granuloma. The initial study, conducted in rhesus, was then validated in cynomolgus macaques, where the effect of BCG vaccination on the progression of granuloma gene expression was also studied (Mehra et al. 2013). These studies indicated that primate lung TB granulomas were characterized by a high degree of expression of markers of adaptive immune dysfunction, such as indoleamine 2,3-dioxygenase (IDO), Arg1, LAG-3 and PD-1 (Mehra et al. 2013). Many of these observations have since been validated (Mattila et al. 2013; Phillips et al. 2015; Gautam et al. 2017). These results suggest that either immune exhaustion or suppression events may be common in granulomas. In support of this, T cells derived from NHP TB granulomas appear to be dysfunctional (Gideon et al. 2015), although it has recently been suggested that exhaustion may not be the main mechanism for this (Wong et al. 2018). Inhibition of IDO activity relieves the inhibition on T cells (Gautam et al. 2017) and it may soon be possible to test the impact of inhibiting other immune checkpoints, e.g. LAG-3 and PD-1 in this model, using either an antibody/depletion or inhibitor/antagonism approach. Numerous other cellular pathways that have been identified to be involved in TB pathogenesis using murine models of TB can be validated using NHPs to assess the feasibility of designing host-directed therapies directed against these pathways. These include the C-Abl pathway (Napier et al. 2011, 2015), the SIRT pathway (Cheng et al. 2017) and the AMPK pathways (Singhal et al. 2014). The C-Abl pathway can be modulated by the inhibitor Imatinib, a tyrosine kinase inhibitor (TKI). Inhibition of the C-Abl pathway by Imatinib correlates with better control of TB via myelopoiesis (Napier et al. 2011, 2015). The SIRT pathway can be induced using agonists like pterostilbene (PTS) and its induction correlates with better control of TB due to enhanced macrophage function (Cheng et al. 2017). The AMPK pathway can be induced by the antidiabetic drug metformin (Singhal et al. 2014). Several of these pathways suggest the interface between immunity and cellular metabolism during *Mtb* infection. *Mtb* is already well described to preferentially alter the host's cellular metabolism, in addition to its own metabolism. Furthermore, these pathways also suggest mechanisms by which T2D and other metabolic syndromes could affect the immunity to TB in individual patients.

System-wide transcriptomic approaches in NHPs infected with *Mtb* have also been used to identify correlates of risk of TB development in the blood (Gideon et al. 2016) as well as lung tissue (Kaushal et al. 2015). These results show that transcriptional changes in this model translate well to the human situation and can be used for novel discoveries. In studies of macaques

infected with specific *Mtb* mutants, the global host response has identified correlates of lack of disease despite infection (these responses may or may not translate to correlates of protection) (Mehra et al. 2012). By investigating global responses in the lungs of rhesus macaques infected with *Mtb*: Δ *dosR* mutants which are deficient in responding to hypoxia, we have found that *Mtb* actively subverts the recruitment of Th1 cells into the early granuloma (Mehra et al. 2015). System-wide measurements have also been applied to the granuloma level in both rhesus (Mehra et al. 2010) and cynomolgus (Mehra et al. 2013) macaques. In both models, we conclude that early lesions are characterized by a proinflammatory cytokine storm but later lesions are reprogrammed to suppress such responses (Mehra et al. 2010). It has also been found that a balance of pro- and anti-inflammatory cytokines, including IL-10 and IL-17, are associated with sterilization (Gideon et al. 2015). We have since successfully used lung and bronchoalveolar lavage (BAL) responses to study vaccine-induced protection (Kaushal et al. 2015), as well as the effect of host-directed therapeutics (Gautam et al. 2017).

Some of the recent advances in the NHP model have resulted from the use of high-definition live imaging using [18 F]2-fluorodeoxy-D-glucose ([18 F]FDG) positron emission tomography-computed tomography (PET/CT) scanning (Lin et al. 2013; Coleman et al. 2014; Lin et al. 2014; Martin et al. 2017; White et al. 2017). PET has proven particularly useful in determining the inflammatory status of individual lesions. Generally, these studies suggest that PET-hot granulomas are prevalent in early time points and later time points are characterized by increased PET-cold lesions (Coleman et al. 2014). These studies confirm previous transcriptomics results that showed a cooling of proinflammatory signatures in granulomas over the course of time (Mehra et al. 2010, 2013). The most important message from these studies is the extensive heterogeneity in granuloma phenotype. Each granuloma appears to be an individual entity and factors governing their inflammatory status are not well understood. Recent cutting-edge molecular studies in this model include the use of digitally barcoded strains (Martin et al. 2017) to identify the founder bacilli for individual lesions and blockade of individual immune pathways and cells (Diedrich, Mattila and Flynn 2013; Gautam et al. 2017). Some of the recent advances in anti-TB vaccine discovery have involved the use of isogenic *Mtb* mutants (Kaushal et al. 2015; Foreman et al. 2017) or specific cytomegalovirus-based viral vectors in this model.

MICE

Rodent, and primarily murine, research is ubiquitous in science and is utilized in the study of nearly every human disease as ~99% of all mouse genes possess a human homolog (Consortium 2002). Mice also have the benefit of posing a low cost to laboratories and having a wide variety of available genetic knockouts for immunological studies. Cost is of particular importance to TB research since the requirement for specialized containment facilities leads to increased expenses to begin with. In terms of an animal model for TB, mice are susceptible to disseminating infection and are typically incapable of controlling the infection (Kramnik, Demant and Bloom 1998). Most strains do not form classical granulomas like those found in humans (see Figure 1) and instead form diffused, non-caseating structures, such as the C57BL/6 strain that forms non-necrotic lesions primarily comprised of neutrophils (Flynn 2006; Kramnik and Beamer 2016). Thus, while the mouse model of TB has been extremely useful in identifying key immune mechanisms responsible for the control of TB (Orme 2003), as well

as for the assessment of drugs, therapeutics and regimens, its utility in effectively modeling the progression of *Mtb* infection, its pathology and the host-pathogen interactions that lead to either finite control or progressive TB disease, has been questioned. It has been suggested that the microenvironment within human TB lesions so profoundly impacts the physiology and the metabolism of the pathogen that the study of TB in systems that do not recapitulate these specific stress conditions (e.g. hypoxia) present within human TB lesions can be fundamentally misleading (Kaufmann et al. 2005). Recently, however, murine systems have been developed that recapitulate specific conditions. For example, granulomas formed in C3HeB/FeJ (Kramnik) mice infected with *Mtb* develop caseating necrosis and become centrally hypoxic (Driver et al. 2012). B6.C3H^{SST1} mice provide stable hypoxic lesions to study (Kramnik 2008) and CBA/J IL-10 KO are able to model mature fibrotic granulomas (Cyktor et al. 2013). Of particular interest also are mouse models of *Mtb*/HIV co-infection that utilize humanized mice. HuMouse develop a progressive, disseminating *Mtb* infection producing caseous granulomatous inflammation (Calderon et al. 2013). Endsley and colleagues showed that increased *Mtb* replication (≥ 1 -log) and larger and diffused lesions are observed in these mice following HIV/*Mtb* co-infection (Nusbaum et al. 2016).

Studies built on the comparison of inbred strains of mice, with varying degrees of susceptibility to *Mtb* infection (Fortin et al. 2007), are vital for deciphering the role of singular changes to the immune response that may have diverse effects on the overall host. These models have been used for multimodal *in vivo* and *ex vivo* imaging to assess necrosis, fibrosis, RNAseq of whole granulomas, macrophage recruitment and death, as well as IFN and ISR pathway activation for comprehensive quantitative evaluation of the dynamics of TB granulomas.

Recently, two additional mouse models have captured the imagination of the field, largely due to their ability to offer maximal allelic variation within a mouse model that more closely resembles human population dynamics. One, known as the Collaborative Cross (CC) model, is a large panel of new inbred mouse strains derived from an eight-way cross using a set of Jackson Labs mice that included three wild-derived strains. CC mice display a broad range of susceptibility to *Mtb* infection, which are expectedly heritable (Smith et al. 2016). The other, known as the Diversity Outbred (DO) model, represents a far more diverse underlying genotype and was produced by a novel outbreeding strategy that maintained a balanced mixture of the founder genomes and avoided allelic loss and inbreeding (Churchill et al. 2012). Each DO mouse is genetically unique and, unlike inbred strains, DO mice have normal levels of heterozygosity, recapitulating that of humans (Svenson et al. 2012). The Jackson Labs DO model was generated using 160 breeding lines as founders, some which were also used to construct the CC mice (Churchill et al. 2012). These DO mice have since been established as the models of choice to study qualitatively different outcomes in behavior (Logan et al. 2013), cancer (Winter et al. 2017) and infectious diseases (McHugh et al. 2013). DO mice display a wide range of pathologic phenotypes following influenza virus or bacterial co-infection (McHugh et al. 2013). These results offer a glimpse of the powerful potential that these newer mouse models, especially the DO outbred model, provide in the study of complex human diseases with varied phenotypes. TB is one such disease, with *Mtb* infection leading to varied outcomes over the course of time. Genetics has been strongly implicated in this diversity of phenotypes. When DO mice were infected with standard doses of *Mtb* via aerosol, super-susceptible, susceptible and resistant phenotypes were readily observed (Niazi et al. 2015).

In addition to experiencing highly variable outcomes, DO mice exhibited a spectrum of necrotic (human-like) lesions including visible necrosis of individual macrophages, necrotizing granulomas with a fibrotic capsule, tuberculous pneumonia with intra-alveolar neutrophilic exudates and thrombosis of the lung capillaries (Niazi et al. 2015; Kramnik and Beamer 2016).

More recently, the DO mouse collection has been used to measure the impact of host-directed therapeutics (specifically vitamin D) on the progression of *Mtb* infection (Bhatt et al. 2016). In these studies, changes in host-specific immune responses, e.g. cellular recruitment and phenotype, its impact on the granuloma structure and changes in bacterial burden could be readily measured (Bhatt et al. 2016). DO mice exhibit a range of bacterial burdens following aerosol infection with *Mtb*, enabling DO mice to act as an improved mouse co-infection model (Monin et al. 2015). The DO mouse model has been able to recapitulate complex phenotypes related to *Mtb* infection, such as helminth co-infections (Monin et al. 2015), as well as vaccine efficacy (Griffiths et al. 2016). Furthermore, several genes in a TB susceptibility signature identified in an adolescent cohort study of human household contacts (Zak et al. 2016) that predicted progression to TB far earlier than when clinical signs were apparent can be detected in the lungs of *Mtb*-infected DO mice, but not conventional B6 mice, by RNAseq (our unpublished data).

In the process of testing other toll-like receptor (TLR) agonists to similarly activate innate DC pathways in mice, we identified monophosphoryl lipid A (MPL) as a safe adjuvant for pulmonary delivery (Ahmed et al. 2017). We next tested whether the delivery of MPL and CD40 agonist FGK 4.5 in DO mice improves *Mtb* control, as seen in inbred B6 mice (Ahmed et al. 2017). Accordingly, individual DO mice were vaccinated with BCG, and at the time of *Mtb* infection received MPL, FGK4.5 along with IL-10R neutralizing antibody. IL-10 was identified as a key anti-inflammatory cytokine that limits T cell responses (Griffiths et al. 2016). These new data show that DO mice that received the DC activation therapy resulted in improved *Mtb* control and formed enhanced B cell follicles, which serve as a correlate of improved *Mtb* control in both murine and NHP animal models (Slight et al. 2013; Kaushal et al. 2015; Foreman et al. 2016). DO mice mimic the spectrum of disease seen in human TB and, similar to humans, exhibit variability in control upon *Mtb* challenge (Gopal et al. 2013). These model systems are, therefore, likely to advance our understanding of TB pathogenesis and immunity in the years to come. Furthermore, these will serve as initial screening tools for therapeutic agents in the setting of human-like genetic variability.

Mice are immensely valuable for their ability to provide a clear analysis of protection in vaccine studies and in mechanistic studies using genetic knockouts. This latter attribute is particularly important since the interaction of the *Mtb* bacillus with the host is complex and multi-parametric. Numerous cell types of the immune system and several immune pathways are involved, giving rise to variability and heterogeneity in phenotypes, which mechanistic approaches can better control.

VALIDATION OF MOLECULAR DETERMINANTS OF INFECTION, DISEASE AND PROTECTION IN THE MURINE MODEL

Seminal murine studies have established principles of *Mtb* infection progression and TB disease in the murine model and identified bacterial determinants of disease and host contributions

into both protection from and pathology of TB. The current iteration of the murine model of TB was optimized in the early 1990s with the advent of the ability to infect mice with aerosols of *Mtb*. These studies showed that *Mtb* was more virulent when the exposure route was aerosol instead of the previously used intravenous route (North 1995). This led to the adoption of the aerosol route of murine infection as the model of choice by the field. Further experimentation with this route of infection showed that, unlike systemic infection with *Mtb*, adaptive immune responses are not initially elicited following *Mtb* infection via the aerosol route (Cooper 2014), a result which has since been confirmed in other experimental models (Mehra et al. 2010).

Using a mouse model where the route of infection is comparable to humans was a defining achievement. Simultaneously, landmark studies by Jacobs, Bloom and others allowed the development of genetic systems for the manipulation and the investigation of *Mtb* (Jacobs et al. 1989, 1990; Cirillo et al. 1991; Jacobs et al. 1991; Balasubramanian et al. 1996). These systems allowed the generation of isogenic mutants of *Mtb* for testing in murine virulence experiments. Eventually, genome-wide libraries of *Mtb* transposon-interrupted mutants were generated which allowed a system-wide investigation of the bacterial genes required for the survival of the pathogen in murine lungs (Sasseti and Rubin 2003; Lamichhane, Tyagi and Bishai 2005). Successful execution of these projects has since allowed a limited number of similar investigations to be performed in guinea pigs and macaques (Jain et al. 2007; Dutta et al. 2010). One approach was to generate Mariner-transposon mutants of *Mtb* with lesions in individual genes and to pool them for use in *in vitro* or *in vivo* studies, known as Designer Arrays for Define Mutant Analysis (DeADMAN). This allowed >1/3rd of all *Mtb* genes to be mutated and individual mutants included in the library (Lamichhane et al. 2003). Bayesian statistical analyses of this library revealed that at least a third of the *Mtb* genes may be essential and it may not be possible to interrupt these. The DeADMAN approach has been used to study molecules required for bacterial survival and persistence in mice (Lamichhane, Tyagi and Bishai 2005). It was found that 6% of the *Mtb* mutants were essential for growth in the mouse model, including those encoding for mycobacterial membrane large and small protein family (mmpL/S), as well as genes involved in the synthesis of (p)ppGpp, the alarmone that signals stringent response due to stress and amino acid starvation (Haseltine and Block 1973). Most of these observations have since been confirmed using specific isogenic mutants and the molecules implicated in these murine screens have been validated to be critical for TB disease progression (Varela et al. 2012; Weiss and Stallings 2013; Degiacomi et al. 2017). Another approach that was used to test the consequences of genome-wide mutagenesis of *Mtb* genes in the mouse model was Transposon Site Hybridization (TraSH). This approach combined high-density insertional mutagenesis with either microarray- or TnSeq-based mapping of mutant pools enriched or diminished post-infection (Sasseti, Boyd and Rubin 2001; Sasseti and Rubin 2003; Griffin et al. 2011, 2012; DeJesus et al. 2017). These studies identified a core group of ~200 genes (5% of the genome, a number that compares favorably to the one arrived at by the DeADMAN approach), which were required for the survival of *Mtb* in mice. Many of these genes were unique to mycobacteria and the majority were of hitherto unknown functions (Sasseti and Rubin 2003). Many of the core genes required for virulence in the mouse model have since been individually validated to encode molecules important for *in vivo* survival and/or persistence of the bacillus.

Since the first draft of the *Mtb* genome sequence became available, it has become abundantly clear that this remarkable pathogen has evolved to respond to a variety of stress conditions during its *in vivo* life cycle (Cole et al. 1998). Numerous stress conditions that could be biologically relevant *in vivo* have been identified, e.g. damage to *Mtb* cell wall, or DNA, acidic pH in the phagosomal environment, nitrosative and oxidative stresses, nutritional starvation including phosphate, and hypoxia (Stallings and Glickman 2010). These stressors do not work in isolation but may in fact be interlinked. Furthermore, *Mtb* genes and pathways have been implicated to be involved in defense against such stress conditions and specific knock-out of molecules or pathways analyzed for phenotype relative to wild-type *Mtb* in the mouse model of aerosol infection. Thus, a mutant in the *kasB* gene, which encodes an enzyme in the mycolic acid synthesis pathway, was susceptible to treatment with antimicrobial peptides that damage cell wall and found to be highly susceptible to killing in the murine model. These results showed a connection between the specific molecular function of an *Mtb* protein and its ability to encounter and respond to *in vivo* relevant stress. Numerous other mutants in genes involved in cell wall component synthesis, maintenance and modification (e.g. phthiocerol dimycocerosate, mycolic acids and cyclopropanes) have been observed to be deficient for survival in murine lungs (Glickman, Cox and Jacobs 2000; Rao et al. 2005). Similarly, mutants in *Mtb* genes that protect against or repair DNA damage also have significant defects for growth in the murine model. For example, Rv1633, which encodes for UvrB, forms a critical part of the nucleotide excision repair machinery (Darwin et al. 2003). Interestingly, there was a significant defect in the killing of mice by a strain unable to synthesize UvrB, but the difference in tissue bacterial burdens was minimal (Darwin et al. 2003). These results suggest that while the murine model may be good for replicating several stress conditions that *Mtb* faces in human lungs, it is possible that specific mechanisms that restrict the replication of the bacillus in human lungs may be somewhat differently invoked in murine lungs. *Mtb* mutants in serine protease genes Rv3671c and Rv2224c were unable to survive in murine lungs, validating their role in protecting the bacillus from acidic pH by preserving intrabacillary pH (Vandal et al. 2008; Vandal, Nathan and Ehrh 2009). *Mtb* uses a multitude of pathways to counter oxidative burst of the phagosome, indicating the importance of being able to survive in the wake of this stress. These include a catalase gene *katG* (Pym, Saint-Joanis and Cole 2002) and two superoxide dismutase genes, *sodA* (Edwards et al. 2001) and *sodC* (Piddington et al. 2001). Mutants in *katG* have strong defects in murine growth (Pym, Saint-Joanis and Cole 2002) and *sodA* is an essential gene. When the expression of *sodA* was perturbed by antisense RNA approach, the resulting strain survived poorly in mice (Edwards et al. 2001). *Mtb* also experiences oxidoreductive stress and has extensive means to respond to it. *Mtb* encodes a stress response network orchestrated by an alternate, extracytoplasmic factor response sigma factor *sigH*, which is induced in response to reductive, acidic pH, heat shock and a variety of other stresses (Fernandes et al. 1999; Manganelli et al. 1999). SigH orchestrates an antioxidative response by inducing the expression of thioredoxin/thioredoxin reductase proteins (Bell et al. 2016). Surprisingly, a mutant in the *sigH* gene, while being susceptible to oxidoreductive stress, could grow in mice to levels comparable with wild-type *Mtb* despite evidence of lesser pathology (Kaushal et al. 2002).

The murine model has perhaps been most beneficial in establishing the key role of various components of the immune system in protection from TB. Thus, seminal studies by Flynn

and Bloom, and Cooper and Orme, established the importance of CD4⁺ T cells in protecting from TB, especially those expressing IFN- γ and IL-12 and those involved in TNF- α induction (Cooper et al. 1993; Flynn et al. 1993a, 1993b, 1995a, 1995b; Cooper et al. 1997). Since then, mouse studies have revealed a relatively smaller role of CD8⁺ T cells in protection from *Mtb* infection (Lin and Flynn 2015) and an even more miniscule contribution of B cells and humoral immunity (Chan et al. 2014). Work by Khader and Cooper has since revealed an interesting role for the Th17 branch of immunity in TB (Khader et al. 2005, 2007). More recent work suggests that the role of some immune pathways, particularly Th17, may be governed by the strain of *Mtb* used and its relative pathogenicity (Gopal et al. 2014). Similarly, Turner and colleagues have established the negative role of IL-10 signaling in the control of *Mtb* infection (Beamer et al. 2008). These studies have since allowed us to focus on the function of specific lymphocytes. Thus, it has been postulated that murine granuloma T cells may have compromised cytokine secretion and functional activity (Egen et al. 2008; Bold et al. 2011; Egen et al. 2011).

RABBITS

Early on, rabbits of different origins were shown to have differential susceptibility to TB (Lurie, Zappasodi and Tickner 1955). Rabbits have commonly been infected via the aerosol route (Manabe et al. 2008), however human strains of *Mtb* are not fully virulent in rabbits (Dannenberg and Collins 2001). During infection with *M. bovis*, rabbits are able to form pulmonary cavitation, which has been considered to resemble *Mtb*-induced granulomas in humans more faithfully than those formed in mice and guinea pigs (Dannenberg 2006). Most of the species available today are resistant to *Mtb* as Lurie's susceptible breed had died out (Dharmadhikari and Nardell 2008). Seminal studies by Dannenberg et al. showed that alveolar macrophages are the target cell for *Mtb* and are required for resistance to TB (Henderson, Dannenberg and Lurie 1963; Rojas-Espinosa et al. 1974). Resistance to TB required proteolytic enzymes functioning in the endocytic pathway (Henderson, Dannenberg and Lurie 1963; Rojas-Espinosa et al. 1974). Dannenberg also illuminated the phenomenon of delayed type hypersensitivity in the rabbit model (Dannenberg 1991). More recently, the model has been employed to study mechanisms of *Mtb* persistence and reactivation (Geiman et al. 2004; Kesavan et al. 2009), cavitary dissemination (Nedelchev et al. 2009), response of *Mtb* to host-mediated hypoxia in the granuloma (Converse et al. 2009), correlates of immune-mediated latency (Subbian et al. 2013a, 2013b) and both the environment of failed immunity and its correction by host-directed therapeutics (Subbian et al. 2011a, 2011b, 2016). Manabe et al. have developed the only animal model of TB-related immune reconstitution inflammatory syndrome (IRIS) in rabbits to study the progression and severity of the immune reconstitution inflammatory syndrome (Manabe et al. 2008). The model has now been employed for testing drug effectiveness, particularly pharmacokinetics and pharmacodynamics, to estimate drug penetration into different types of lesions in experimentally infected rabbits (Rifat et al. 2018). Rabbits also form caseating, necrotic granulomas (Figure 1) which, under the correct conditions, may liquefy and cavitate (Nedelchev et al. 2009). Bishai and colleagues have developed a reliable model of cavitary disease in rabbits and confirmed pathological and molecular correlates of human disease. This model permits *in vivo* imaging to observe and quantify the events leading to cavitation (Kubler et al. 2015) and thus has tremendous potential in the study of granuloma breakdown.

VALIDATION OF MOLECULAR DETERMINANTS OF INFECTION, DISEASE AND PROTECTION IN THE RABBIT MODEL

As discussed above, Subbian *et al.* have characterized various outcomes of *Mtb* infection in the rabbit model (Subbian *et al.* 2011a, 2012, 2013b; Tsenova *et al.* 2014). They also applied whole genome transcriptomic evaluation to better understand universal host responses as they relate to the progression of infection (Subbian *et al.* 2012). Rabbits infected with low doses of low-virulence strain CDC1551, which elicits strong immune responses, initially exhibited bacterial growth and pathology, but eventually controlled infection in a latent form (Subbian *et al.* 2012). Genome-wide transcriptional analysis supported this notion, with the number of genes involved in both inflammatory responses and in lung remodeling (required for granuloma formation) peaking 2 weeks after infection in rabbit lungs and then progressively reducing in extent (Subbian *et al.* 2012). Similar results were earlier obtained in both the rhesus (Mehra *et al.* 2010) and the cynomolgus (Mehra *et al.* 2013) macaque models, which is discussed subsequently. The use of PET/CT imaging was first employed in the rabbit model of TB to study the dynamics of infection. These studies also confirmed earlier transcriptomic outcomes that low-dose experimental infection in this model involved an initial inflammatory phase followed by a chronic phase with reduced granuloma-associated inflammatory signal (Via *et al.* 2012). Thus, the use of state-of-the-art molecular approaches in animal models have extended our understanding of the granuloma formation/maturation kinetics in a manner not possible in humans. Circulatory human cytokine/chemokine profile-based molecular analytes are now being used to successfully study *Mtb* infection in rabbits (Dehnad *et al.* 2016).

Transcriptomics has also been employed in the rabbit model to successfully evaluate responses to therapy, including host-directed therapy (Subbian *et al.* 2011a). However, the most striking use of global 'omics' approach in this model has been to compare infection with a high-immunogenic vs. a high-virulence strain of *Mtb* (Subbian *et al.* 2013b). According to these reports, *Mtb* CDC1551, and perhaps other hyper-immunogenic strains, express early lung transcriptional profiles of infection that result in reduced STAT1 induction and polymorphonuclear leukocyte (PMN) recruitment and inflammation. Hence, the usual outcome of infection in rabbits with this strain is LTBI (Bishai *et al.* 1999; Subbian *et al.* 2012, 2013b). On the contrary, STAT1 and inflammatory signals were highly expressed following comparable infection with the hypervirulent HN878 strain and led to high levels of PMN recruitment and cavitary disease (Subbian *et al.* 2013b). While further research across experimental models is necessary, these results provide a potential mechanism for how hyper-virulent strains may have acquired the ability to elicit lung pathology as a means of overcoming the ability of the large majority of human beings to restrict *Mtb* infection as LTBI.

GUINEA PIGS

Guinea pigs are susceptible to *Mtb* and form granulomas, so they have been useful in immunohistopathological stages of pulmonary granuloma formation (Dannenberg and Collins 2001; Turner, Basaraba and Orme 2003). Guinea pigs have also been useful in investigations of responses to antigenic lipopeptides due to their possession of type I CD1 presentation molecules (Kaufmann *et al.* 2016). Initially considered the gold standard for vaccine testing against TB, the guinea pig model is considered highly susceptible (Clark, Hall and Williams 2014) and

has suffered from a lack of immunological tools such as guinea pig-specific antibodies for immune analysis in the past. It is expected, however, that more tools will be available in the future. Thus, a genome-wide interrogation of transcriptional responses to *Mtb* infection in this model is now possible (Jain, Dey and Tyagi 2012). An immune-gene specific array platform has been available for over a decade now and has been used to study *Mtb* infection in this model (Tree *et al.* 2006). This reagent has validated that *Mtb* infection of guinea pigs elicits strong and immediate Th1 responses (Tree *et al.* 2006), indicating its ability to recapitulate aspects of the human immune response to *Mtb*.

VALIDATION OF MOLECULAR DETERMINANTS OF INFECTION, DISEASE AND PROTECTION IN THE GUINEA PIG MODEL

Guinea pigs have been successfully leveraged to study *Mtb*-specific metabolic fate. With the increasing understanding that the immune system interacts with metabolism with influential consequences on the fate of various infections, interest in the guinea pig model of TB has also increased. Recently, important studies have investigated persisters in lung granulomas of this animal model (Lenaerts *et al.* 2007). Basaraba *et al.* have recently been successful in developing a model of TB/T2D in these guinea pigs (Podell *et al.* 2014, 2017). This is an exceptionally significant advance. T2D is a major cause of morbidity and mortality worldwide amongst non-infectious causes and accounts for ~90% of the entire diabetic burden (Deshpande, Harris-Hayes and Schootman 2008). Furthermore, the rates of T2D have been significantly increasing both globally as well as in areas of the world where TB is endemic. Thus, globally, it is predicted that the number of T2D patients will double between 2010 and 2030 (Rowley *et al.* 2017). It has been proposed that T2D is a comorbidity that is fueling the TB pandemic in certain parts of the world at levels comparable with HIV co-infection, especially in regions where TB is already endemic (Dooley and Chaisson 2009). When T2D was induced in guinea pigs, *Mtb* infection caused a significantly higher degree of TB disease with reduced survival and severe pathological presentation than controls (Podell *et al.* 2014). This coincided with uncontrolled inflammatory responses. Thus, the guinea pig model allows an in-depth study of T2D/TB comorbidity, including the testing of novel approaches for therapeutic control.

The guinea pig model has also been used in mutant infection screening experiments to better understand the bacillary determinants of pathogenesis (Jain *et al.* 2007). When compared with mice infected with the identical mutant library, guinea pigs permitted an accelerated detection of genes required for the *in vivo* survival of *Mtb* (Jain *et al.* 2007). These results suggest that TB-related pathology in guinea pig lungs results in relatively greater immune pressure on the bacillus than the corresponding murine pathology.

RATS

Rats remain a more recent and less developed model of TB infection out of all mammalian models. Recently, Lewis rats (Sugawara, Yamada and Mizuno 2004a), American cotton rats (Elwood *et al.* 2007), Wistar rats (Gaonkar *et al.* 2010; Cardona *et al.* 2011; Singhal *et al.* 2011) and diabetic rat strains (Sugawara, Yamada and Mizuno 2004b; Sugawara and Mizuno 2008) have been used to develop the rat model of TB infection. They have also been used to demonstrate Th1-shifts in immunity

following BCG vaccination (Cardona et al. 2011). Unlike mice, rats have been found to form well-organized granulomas and accumulate foamy macrophages (Figure 1), which have been associated with pulmonary lesion resolution (Singhal et al. 2011). Rats have been used successfully as an animal model for testing drug efficacy (Gaonkar et al. 2010; Foo et al. 2011) and antigen-specific T cell responses (Cardona et al. 2011; Foo et al. 2011; Singhal et al. 2011) and have been a valuable animal model for pharmacokinetic and toxicology studies by pharmaceutical companies (Gumbo et al. 2015). Similar to NHP models of TB infection, Wistar rats exposed to a low dose infection were found to control or clear bacilli by 4 months post-infection, depending on the strain used (Singhal et al. 2011), indicating that they have the potential to model latency. As a co-infection model, rats would be of benefit in the exploration of diabetes as Goto Kakizaki T2D rats (McGillivray et al. 2014) and Komeda diabetes-prone rats (Sugawara and Mizuno 2008) have been shown to have a higher susceptibility to TB compared with wild-type rats. Like mice, rats are simple to house, however they can withstand repeated blood collections and could be used during long-term infection studies.

ZEBRAFISH

Despite the fact that zebrafish do not possess lungs, researchers have been successful in utilizing their infection with *M. marinum* to uncover the mechanisms of microgranuloma formation prior to antigen-specific responses (Volkman et al. 2010; Ramakrishnan 2013). This model is useful in the determination of elementary mycobacterial pathogenesis and treatment. Many seminal recent discoveries about granuloma induction and the role of various mycobacterial virulence factors in this process have been initiated in this model (Volkman et al. 2010; Cambier et al. 2014). Zebrafish were vital in showing that macrophage death spreads bacilli to adjacent macrophages encapsulated within the same granuloma (Volkman et al. 2004). Many of these observations were extended from the fish model and validated in clinical human samples (Tobin et al. 2010). Recently, investigations in this system have identified specific mechanisms by which granulomas mature, with specialized phagocyte morphology contributing to the pathology of the lesion and promoting bacillary replication and persistence (Cronan et al. 2016). The zebrafish embryo model was used to show innate immune mechanisms that lead to granuloma formation, including induction of innate immune aggregates (Figure 1) and activation of *M. marinum* granuloma-specific genes (Davis et al. 2002). Zebrafish are small, easy to image (especially in the larval stage where the lesions can be directly visualized) and offer the advantage of significantly powered group sizes while posing minimal increases in overall cost (Cronan and Tobin 2014). The lack of host homology, however, limits the ability of zebrafish to completely model human infection, particularly latency. Special facilities are required for studies utilizing the fish model, however these studies have an additional benefit of being conducted at BSL2 since most studies utilize the strain *M. marinum*, which rarely infects humans, rather than *Mtb*.

IMPORTANCE OF ANIMAL MODELS FOR DRUG AND VACCINE EFFICACY

Animal models are important for evaluating new drug regimens prior to introduction in humans. With humans, the threat of

developing drug or multidrug resistant strains prevents rapid introduction and animals, particularly mice, provide a niche where drug regimens can be used quickly to determine if new regimens are efficacious in treating and eliminating TB. Mice were useful in showing that rifapentine and moxifloxacin could be used to eliminate TB within 2 months, rather than the standard 6 months with rifampin (R), isoniazid (H) and pyrazinamide (Z) (Rosenthal et al. 2007). The use of different strains is vital to the evaluation of drug efficacy for later human use. Dual and triple drug combinations of H, R and Z have shown that different challenge strains yielded different *in vivo* efficacy in BALB/c mice (De Groote et al. 2012), leading to the need to develop improved drug regimens for eventual human use. Animal models allow an avenue to explore these new drugs prior to human use. Different quinolones (Lenaerts et al. 2008) and nitroimidazo-oxazines (Nuermberger et al. 2008) have recently been investigated to replace the current three-drug regimen. Recently, a subclass of quinolones called 2-pyridones has been investigated, including KRQ-10 018, and was found to be similar in *in vivo* efficacy to isoniazid and have less activity than moxifloxacin (Lenaerts et al. 2008). When the nitroimidazo-oxazine PA-824 was used with moxifloxacin and pyrazinamide regimen as a substitute for rifapentine, it cured mice more quickly than the standard regimen, but not within 3 months of treatment (Nuermberger et al. 2008).

As early as 1997, it was suggested that vaccine efficacy is altered based on the number of passages that the BCG strain has undergone (Behr and Small 1997). Behr et al. found that significant genetic changes had accumulated in many of the BCG strains, including the loss of region of difference 2 (RD2) in BCG-Denmark, BCG-Tice and BCG-Glaxo (Behr and Small 1999). In mice, it was found that the Prague and Japanese strains of BCG had lower recovery rates compared with three other BCG strains and were unable to protect against a second challenge with *Mtb* (Lagranderie et al. 1996). These studies highlight that, despite the presence of a vaccine that has been accepted for a long period, proper maintenance of the vaccine strain is vital in ensuring its efficacy. Experiments in animal models are thus necessary to ensure that efficacy is maintained over time to protect vulnerable human populations.

It has been observed for some time that BCG is primarily efficacious in the prevention of disseminated TB in children, so studies in other animal models are vital in investigating improvements that must be made to improve protection. Animal models have supported the lack of widespread protection produced by the BCG vaccine. Using *Mtb* Erdman and HN878 challenge to evaluate parenteral BCG vaccination efficacy, Verreck et al. showed that protection widely varies in rhesus macaques, as is the case in humans (Verreck et al. 2017). Until we possess a vaccine that prevents adult pulmonary TB, it will be impossible to eliminate the disease. Due to the existence of multidrug and extremely drug-resistant TB, treatment improvements alone will not be sufficient to contain the infection. Improved developments in vaccines, treatments and patient support for improved compliance will be necessary to ultimately eliminate TB.

IMPORTANCE OF STRAIN-STRAIN COMPARISON IN ANIMAL MODELS

It has been estimated that humans can be infected with *Mtb* with an infectious dose as low as 10 CFU. Humans have a 10% lifetime

risk of progressing to active disease once they have acquired TB (Society AT 2000). As a result, using an animal model with a higher susceptibility rate would make sense in replicating the natural, human response to infection. As humans have different susceptibilities to different strains of *Mtb*, it is ideal to compare the susceptibilities of each animal model with different strains of *Mtb*, as well as using multiple strains of *Mtb* to model treatment efficacy (De Groote et al. 2012). Several animal models have been used for side-by-side susceptibility comparisons of different strains such as *Mtb* Erdman, H37Rv and CDC1551 including mice (Kelley and Collins 1999; Manca et al. 1999; Ordway et al. 2007; De Groote et al. 2012), rabbits (Manabe et al. 2003; Tsenova et al. 2005), rats (Singhal et al. 2011) and NHPs (Cadena et al. 2016). In early comparisons over the course of 3 months, mouse studies failed to show differential susceptibilities among different strains of *Mtb* in intravenous or aerosol challenge (Kelley and Collins 1999). A study involving comparisons of rabbits aerosol-challenged with *Mtb* Erdman, CDC1551 and H37Rv found that Erdman was the most virulent strain and caused a broader spectrum of disease within 16–20 weeks post-challenge (Manabe et al. 2003). Several studies of strain-strain comparisons have been conducted within the NHP model as well. Despite marmosets having high susceptibility, they have been used to reveal that strain and infectious dose can lead to heterogeneous responses by comparing low-dose Erdman and CDC1551 infection ranging from 1–12 CFU (Cadena et al. 2016).

The use of animal models in strain-strain comparison are beneficial in that they enable one to investigate both differences in the host's responses to the individual strains as well as differential virulence mechanisms of the strains themselves. Using New Zealand white rabbits, Tsenova et al. found that phenolic glycolipid (PGL) biosynthesis is critical in subverting the host's ability to produce protective TNF- α by comparing rabbits infected with CDC1551, HN878 and HN878pks1–15::hyg (Tsenova et al. 2005). Using CDC1551 and HN878 infection, Subbian et al. showed that differential leukocyte activation and recruitment within the first 3 h of infection led to vastly different outcomes despite identical infectious doses being used (Subbian et al. 2013b). HN878-infected rabbits that developed cavitory disease had increased expression of pro-inflammatory genes that are associated with extravasation and activation of PMNs in the lungs, including those producing TNF- α , IL-8, IL-15, MCP-1 and CXCL10. Other studies have found that CDC1551 induces a more rapid and robust response in mice, with granulomas containing high levels of TNF- α , IL-6, IL-10, IL-12 and IFN- γ mRNA (Manca et al. 1999). Mice were also vital in showing that some of HN878 hypervirulence is due to the induction of stronger Th1 responses and rapid Treg emergence compared with other strains that result in their decreased survival (Ordway et al. 2007). Studies utilizing multiple challenge strains are also vital in evaluating the protection offered by different vaccines. BCG has shown efficacy in protecting mice from various strains of *Mtb*, however these differ incredibly from clinical observations in humans (Jeon et al. 2008).

ADDITIONAL GENOMIC APPROACHES IN THE ANIMAL MODELS OF TB

The availability of genome sequences in the past two decades has spawned the sciences of systems biology, where the understanding of the entire genome is leveraged to understand the responses of both the pathogen and the host on a 'system-wide' level. Initial studies focused on a simplistic design where

the response of *Mtb* to various *in vitro* conditions directly or indirectly corresponding to stresses that the pathogen might be subjected to *in vivo* were measured. These experiments clearly illuminated that *Mtb* has developed an intricate network of transcriptional networks to fine-tune responses to different stresses. Key molecules in this network included sigma factors, which serve to guide the RNA polymerase to specifically initiate gene expression. The *Mtb* genome encodes about a dozen stress- or condition-specific sigma factors whose expression are often intricately controlled at multiple levels (Gomez, Chen and Bishai 1997). Thus, it was shown that *sigE* responds to stress that weakens the *Mtb* cell wall and, unlike wild-type *Mtb*, Δ *sigE* mutants are effectively killed by phagocytes (Manganelli et al. 2001). *SigE* is one of the few molecules known to be expressed in *Mtb*-infected macrophages by selective capture of transcribed sequences (SCOTS), an early genome-wide RNA measurement technique. Since those early days, the animal models of TB have progressed tremendously, and state-of-the art techniques are now routinely used in these to study both the pathogenesis of *Mtb* as well as the evaluation of host responses involved in immunity as well as pathology. These include comparative genomics, post-genomic mutations, transcriptomics (beginning with DNA microarrays, but now including RNAseq and single cell RNAseq), proteomics (including to detect proteins with modifications such as phosphorylation, etc.), lipidomics, metabolomics and whole animal live imaging by magnetic resonance imaging (MRI) and PET/CT.

CONCLUSIONS

No one animal model recapitulates the breadth of heterogeneity in human TB. However, several new mouse models can portray specific events in the granuloma such as hypoxia or fibrosis. Similarly, guinea pigs, zebrafish and rabbit infection models have significantly contributed to our understanding of *Mtb* infection and the resulting immune response. Perhaps the best recapitulation of *Mtb*-specific pathogenesis events and immunity occurs in NHPs. *Mtb*-infected macaques have been used for transcriptome-wide immune response evaluation in peripheral blood to back-translate key findings for humans. These studies have been extended to evaluate BAL and lung samples. State-of-the-art molecular imaging tools such as PET/CT have been successfully employed in this model. The use of bar-coded strains and isogenic mutants to better understand TB pathogenesis has revealed interesting information from this model. Finally, the use of the NHP model is informing vaccine and therapeutic discovery critical to the future control of the TB pandemic. Overall, the use of animal models improves our understanding of *Mtb* disease pathogenesis and provides us with models that recapitulate aspects of human disease for the testing of new vaccine and treatment regimens that are vital to ultimately eliminate TB.

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REFERENCES

- Ahmed M, Jiao H, Domingo-Gonzalez R et al. Rationalized design of a mucosal vaccine protects against *Mycobacterium tuberculosis* challenge in mice. *J Leukoc Biol* 2017;**101**:1373–81.
- Ai JW, Ruan QL, Liu QH et al. Updates on the risk factors for latent tuberculosis reactivation and their managements. *Emerg Microbes Infect* 2016;**5**:e10.
- Anacker RL, Brehmer W, Barclay WR et al. Superiority of intravenously administered BCG and BCG cell walls in protecting rhesus monkeys (*Macaca mulatta*) against airborne tuberculosis. *Z Immunitätsforsch Exp Klin Immunol* 1972;**143**:363–76.
- Balasubramanian V, Pavelka MS, Jr., Bardarov SS et al. Allelic exchange in *Mycobacterium tuberculosis* with long linear recombination substrates. *J Bacteriol* 1996;**178**:273–9.
- Barclay WR, Anacker RL, Brehmer W et al. Aerosol-induced tuberculosis in subhuman primates and the course of the disease after intravenous BCG vaccination. *Infect Immun* 1970;**2**:574–82.
- Barclay WR, Busey WM, Dalgard DW et al. Protection of monkeys against airborne tuberculosis by aerosol vaccination with bacillus Calmette-Guerin. *Am Rev Respir Dis* 1973;**107**:351–8.
- Beamer GL, Flaherty DK, Assogba BD et al. Interleukin-10 promotes *Mycobacterium tuberculosis* disease progression in CBA/J mice. *J Immunol* 2008;**181**:5545–50.
- Behr MA, Small PM. Has BCG attenuated to impotence? *Nature* 1997;**389**:133–4.
- Behr MA, Small PM. A historical and molecular phylogeny of BCG strains. *Vaccine* 1999;**17**:915–22.
- Bell LC, Pollara G, Pascoe M et al. In vivo molecular dissection of the effects of HIV-1 in active tuberculosis. *PLoS Pathog* 2016;**12**:e1005469.
- Bhatt K, Rafi W, Shah N et al. 1,25 (OH)₂D₃ treatment alters the granulomatous response in *M. tuberculosis* infected mice. *Sci Rep* 2016;**6**:34469.
- Bishai WD, Parrish AM, Jr., Ruiz N et al. Virulence of *Mycobacterium tuberculosis* CDC1551 and H37Rv in rabbits evaluated by Lurie's pulmonary tubercle count method. *Infect Immun* 1999;**67**:4931–4.
- Bold TD, Banaei N, Wolf AJ et al. Suboptimal activation of antigen-specific CD4⁺ effector cells enables persistence of *M. tuberculosis* in vivo. *PLoS Pathog* 2011;**7**:e1002063.
- Brenchley JM, Schacker TW, Ruff LE et al. CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;**200**:749–59.
- Brown JN, Palermo RE, Baskin CR et al. Macaque proteome response to highly pathogenic avian influenza and 1918 reassortant influenza virus infections. *J Virol* 2010;**84**:12058–68.
- Cadena AM, Klein EC, White AG et al. Low dose *Mycobacterium tuberculosis* yield diverse host outcomes in common marmosets (*Callithrix jacchus*). *Comp Med* 2016;**66**:412–9.
- Calderon VE, Valbuena G, Goez Y et al. A humanized mouse model of tuberculosis. *PLoS One* 2013;**8**:e63331.
- Calenda G, Villegas G, Barnable P et al. MZC gel inhibits SHIV-RT and HSV-2 in macaque vaginal mucosa and SHIV-RT in rectal mucosa. *J Acquir Immune Defic Syndr* 2017;**74**:e67–74.
- Cambier CJ, Takaki KK, Larson RP et al. *Mycobacteria* manipulate macrophage recruitment through coordinated use of membrane lipids. *Nature* 2014;**505**:218–22.
- Capuano SV, 3rd, Croix DA, Pawar S et al. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. *Infect Immun* 2003;**71**:5831–44.
- Cardona P-J, Mathys V, Kiass M et al. BCG induces protection against *Mycobacterium tuberculosis* infection in the Wistar rat model. *PLoS One* 2011;**6**:e28082.
- Chackerian AA, Alt JM, Perera TV et al. Dissemination of *Mycobacterium tuberculosis* is influenced by host factors and precedes the initiation of T-cell immunity. *Infect Immun* 2002;**70**:4501–9.
- Chan J, Mehta S, Bharrhan S et al. The role of B cells and humoral immunity in *Mycobacterium tuberculosis* infection. *Semin Immunol* 2014;**26**:588–600.
- Chen CY, Huang D, Wang RC et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. *PLoS Pathog* 2009;**5**:e1000392.
- Cheng CY, Gutierrez NM, Marzuki MB et al. Host sirtuin 1 regulates mycobacterial immunopathogenesis and represents a therapeutic target against tuberculosis. *Sci Immunol* 2017;**2**:eaaj1789.
- Churchill GA, Gatti DM, Munger SC et al. The diversity outbred mouse population. *Mamm Genome* 2012;**23**:713–8.
- Cirillo JD, Barletta RG, Bloom BR et al. A novel transposon trap for mycobacteria: isolation and characterization of IS1096. *J Bacteriol* 1991;**173**:7772–80.
- Clark S, Hall Y, Williams A. Animal models of tuberculosis: guinea pigs. *Cold Spring Harb Perspect Med* 2014;**5**:a018572.
- Cole ST, Brosch R, Parkhill J et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 1998;**393**:537–44.
- Coleman MT, Chen RY, Lee M et al. PET/CT imaging reveals a therapeutic response to oxazolidinones in macaques and humans with tuberculosis. *Sci Transl Med* 2014;**6**:265ra167.
- Consortium MGS. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002;**420**:520–62.
- Converse PJ, Karakousis PC, Klinkenberg LG et al. Role of the dosR-dosS two-component regulatory system in *Mycobacterium tuberculosis* virulence in three animal models. *Infect Immun* 2009;**77**:1230–7.
- Cooper AM. Mouse model of tuberculosis. *Cold Spring Harb Perspect Med* 2014;**5**:a018556.
- Cooper AM, Dalton DK, Stewart TA et al. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* 1993;**178**:2243–7.
- Cooper AM, Magram J, Ferrante J et al. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with mycobacterium tuberculosis. *J Exp Med* 1997;**186**:39–45.
- Cronan MR, Beerman RW, Rosenberg AF et al. Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. *Immunity* 2016;**45**:861–76.
- Cronan MR, Tobin DM. Fit for consumption: zebrafish as a model for tuberculosis. *Dis Model Mech* 2014;**7**:777–84.
- Crostarosa F, Aravantinou M, Akpogheneta OJ et al. A macaque model to study vaginal HSV-2/immunodeficiency virus co-infection and the impact of HSV-2 on microbicide efficacy. *PLoS One* 2009;**4**:e8060.
- Cyktor JC, Carruthers B, Kominsky RA et al. IL-10 inhibits mature fibrotic granuloma formation during *Mycobacterium tuberculosis* infection. *J Immunol* 2013;**190**:2778–90.
- Dannenber AM, Jr. Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. *Immunol Today* 1991;**12**:228–33.
- Dannenber AM, Jr. *Pathogenesis of Human Pulmonary Tuberculosis: Insights from the Rabbit Model*. 2006, Washington, DC: ASM Press.

- Dannenber AM, Jr., Collins FM. Progressive pulmonary tuberculosis is not due to increasing numbers of viable bacilli in rabbits, mice and guinea pigs, but is due to a continuous host response to mycobacterial products. *Tuberculosis* 2001;**81**:229–42.
- Darwin KH, Ehrt S, Gutierrez-Ramos JC et al. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 2003;**302**:1963–6.
- Davis JM, Clay H, Lewis JL et al. Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. *Immunity* 2002;**17**:693–702.
- De Groot MA, Gruppo V, Woolhiser LK et al. Importance of confirming data on the in vivo efficacy of novel antibacterial drug regimens against various strains of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2012;**56**:731–8.
- Degiacomi G, Benjak A, Madacki J et al. Essentiality of mmpL3 and impact of its silencing on *Mycobacterium tuberculosis* gene expression. *Sci Rep* 2017;**7**:43495.
- Dehnad A, Ravindran R, Subbian S et al. Development of immune-biomarkers of pulmonary tuberculosis in a rabbit model. *Tuberculosis (Edinb)* 2016;**101**:1–7.
- DeJesus MA, Gerrick ER, Xu W et al. Comprehensive essentiality analysis of the *Mycobacterium tuberculosis* genome via saturating transposon mutagenesis. *MBio* 2017;**8**:e02133–16.
- Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. *Phys Ther* 2008;**88**:1254–64.
- Dharmadhikari AS, Nardell EA. What animal models teach humans about tuberculosis. *Am J Respir Cell Mol Biol* 2008;**39**:503–8.
- Diedrich CR, Mattila JT, Flynn JL. Monocyte-derived IL-5 reduces TNF production by *Mycobacterium tuberculosis*-specific CD4 T cells during SIV/M. tuberculosis coinfection. *J Immunol* 2013;**190**:6320–8.
- Diedrich CR, Mattila JT, Klein E et al. Reactivation of latent tuberculosis in cynomolgus macaques infected with SIV is associated with early peripheral T cell depletion and not virus load. *PLoS One* 2010;**5**:e9611.
- Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis* 2009;**9**:737–46.
- Douek DC, Brenchley JM, Betts MR et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 2002;**417**:95–8.
- Driver ER, Ryan GJ, Hoff DR et al. 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**:3181–95.
- Dutta NK, Mehra S, Didier PJ et al. Genetic requirements for the survival of tubercle bacilli in primates. *J Infect Dis* 2010;**201**:1743–52.
- Dwivedi VP, Bhattacharya D, Chatterjee S et al. *Mycobacterium tuberculosis* directs T helper 2 cell differentiation by inducing interleukin-1beta production in dendritic cells. *J Biol Chem* 2012;**287**:33656–63.
- Edwards KM, Cynamon MH, Voladri RK et al. Iron-cofactored superoxide dismutase inhibits host responses to *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med* 2001;**164**:2213–9.
- Egen JG, Rothfuchs AG, Feng CG et al. Macrophage and T cell dynamics during the development and disintegration of mycobacterial granulomas. *Immunity* 2008;**28**:271–84.
- Egen JG, Rothfuchs AG, Feng CG et al. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. *Immunity* 2011;**34**:807–19.
- Elwood RL, Wilson S, Blanco JC et al. The American cotton rat: a novel model for pulmonary tuberculosis. *Tuberculosis (Edinb)* 2007;**87**:145–54.
- Fernandes NW, Kong QL, Puyang D et al. A mycobacterial extracytoplasmic sigma factor involved in survival following heat shock and oxidative stress. *J Bacteriol* 1999;**181**:4266–74.
- Flynn JL. Lessons from experimental *Mycobacterium tuberculosis* infections. *Microbes Infect* 2006;**8**:1179–88.
- Flynn JL, Chan J, Triebold KJ et al. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* 1993a;**178**:2249–54.
- Flynn JL, Goldstein MM, Chan J et al. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995a;**2**:561–72.
- Flynn JL, Goldstein MM, Triebold KJ et al. Major histocompatibility complex class I-restricted T cells are necessary for protection against *M. tuberculosis* in mice. *Infect Agents Dis* 1993b;**2**:259–62.
- Flynn JL, Goldstein MM, Triebold KJ et al. IL-12 increases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. *J Immunol* 1995b;**155**:2515–24.
- Foo DG, Tay HC, Siew JY et al. T cell monitoring of chemotherapy in experimental rat tuberculosis. *Antimicrob Agents Chemother* 2011;**55**:3677–83.
- Foreman TW, Mehra S, Lackner AA et al. Translational research in the nonhuman primate model of tuberculosis. *ILAR J* 2017;**58**:151–9.
- Foreman TW, Mehra S, LoBato DN et al. CD4+ T-cell-independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV coinfection. *Proc Natl Acad Sci USA* 2016;**113**:E5636–44.
- Foreman TW, Veatch AV, LoBato DN et al. Nonpathogenic infection of macaques by an attenuated mycobacterial vaccine is not reactivated in the setting of HIV co-infection. *Am J Pathol* 2017;**187**:2811–20.
- Fortin A, Abel L, Casanova JL et al. Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis. *Annu Rev Genomics Hum Genet* 2007;**8**:163–92.
- Franchini G, Nacsa J, Hel Z et al. Immune intervention strategies for HIV-1 infection of humans in the SIV macaque model. *Vaccine* 2002;**20** Suppl 4:A52–60.
- Gandhi NR, Moll A, Sturm AW et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet North Am Ed* 2006;**368**:1575–80.
- Gaonkar S, Bharath S, Kumar N et al. Aerosol infection model of tuberculosis in Wistar rats. *Int J Microbiol* 2010;**2010**:426035.
- Gauld WR, Lyall A. Tuberculosis as a complication of diabetes. *Br Med J* 1947;**1**:677–9.
- Gautam US, Foreman TW, Bucsan AN et al. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2017;**115**:E62–71.
- Geiman DE, Kaushal D, Ko C et al. Attenuation of late-stage disease in mice infected by the *Mycobacterium tuberculosis* mutant lacking the SigF alternate sigma factor and identification of SigF-dependent genes by microarray analysis. *Infect Immun* 2004;**72**:1733–45.
- Gideon HP, Phuah J, Myers AJ et al. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. *PLoS Pathog* 2015;**11**:e1004603.

- Gideon HP, Skinner JA, Baldwin N et al. Early whole blood transcriptional signatures are associated with severity of lung inflammation in cynomolgus macaques with Mycobacterium tuberculosis infection. *J Immunol* 2016;197:4817–28.
- Glickman MS, Cox JS, Jacobs WR, Jr. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of Mycobacterium tuberculosis. *Mol Cell* 2000;5:717–27.
- Gomez JE, Chen JM, Bishai WR. Sigma factors of Mycobacterium tuberculosis. *Tuber Lung Dis* 1997;78:175–83.
- Gopal R, Monin L, Slight S et al. Unexpected role for IL-17 in protective immunity against hypervirulent Mycobacterium tuberculosis HN878 infection. *PLoS Pathog* 2014;10:e1004099.
- Gopal R, Monin L, Torres D et al. S100A8/A9 proteins mediate neutrophilic inflammation and lung pathology during tuberculosis. *Am J Respir Crit Care Med* 2013;188:1137–46.
- Gormus BJ, Blanchard JL, Alvarez XH et al. Evidence for a rhesus monkey model of asymptomatic tuberculosis. *J Med Primatol* 2004;33:134–45.
- Griffin JE, Gawronski JD, Dejesus MA et al. High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. *PLoS Pathog* 2011;7:e1002251.
- Griffin JE, Pandey AK, Gilmore SA et al. Cholesterol catabolism by Mycobacterium tuberculosis requires transcriptional and metabolic adaptations. *Chem Biol* 2012;19:218–27.
- Griffiths KL, Ahmed M, Das S et al. Targeting dendritic cells to accelerate T-cell activation overcomes a bottleneck in tuberculosis vaccine efficacy. *Nat Commun* 2016;7:13894.
- Gumbo T, Lenaerts AJ, Hanna D et al. Nonclinical models for anti-tuberculosis drug development: a landscape analysis. *J Infect Dis* 2015;211 Suppl 3:S83–95.
- Haseltine WA, Block R. Synthesis of guanosine tetra- and pentaphosphate requires the presence of a codon-specific, uncharged transfer ribonucleic acid in the acceptor site of ribosomes. *Proc Natl Acad Sci USA* 1973;70:1564–8.
- Hatzioannou T, Ambrose Z, Chung NP et al. A macaque model of HIV-1 infection. *Proc Natl Acad Sci USA* 2009;106:4425–9.
- Henderson HJ, Dannenberg AM, Jr., Lurie MB. Phagocytosis of tubercle bacilli by rabbit pulmonary alveolar macrophages and its relation to native resistance to tuberculosis. *J Immunol* 1963;91:553–6.
- Henkle E, Winthrop KL. Nontuberculous mycobacteria infections in immunosuppressed hosts. *Clin Chest Med* 2015;36:91–9.
- Huang D, Qiu L, Wang R et al. Immune gene networks of mycobacterial vaccine-elicited cellular responses and immunity. *J Infect Dis* 2007;195:55–69.
- Hulskotte EG, Geretti AM, Osterhaus AD. Towards an HIV-1 vaccine: lessons from studies in macaque models. *Vaccine* 1998;16:904–15.
- Jacobs WR, Jr., Kalpana GV, Cirillo JD et al. Genetic systems for mycobacteria. *Methods Enzymol* 1991;204:537–55.
- Jacobs WR, Jr., Snapper SB, Lugosi L et al. Development of genetic systems for the mycobacteria. *Acta Leprol* 1989;7 Suppl 1:203–7.
- Jacobs WR, Jr., Snapper SB, Lugosi L et al. Development of BCG as a recombinant vaccine vehicle. *Curr Top Microbiol Immunol* 1990;155:153–60.
- Jain R, Dey B, Tyagi AK. Development of the first oligonucleotide microarray for global gene expression profiling in guinea pigs: defining the transcription signature of infectious diseases. *BMC Genomics* 2012;13:520.
- Jain SK, Hernandez-Abanto SM, Cheng QJ et al. Accelerated detection of Mycobacterium tuberculosis genes essential for bacterial survival in guinea pigs, compared with mice. *J Infect Dis* 2007;195:1634–42.
- Jeon BY, Derrick SC, Lim JH et al. Mycobacterium bovis BCG immunization induces protective immunity against nine different Mycobacterium tuberculosis strains in mice. *Infect Immun* 2008;76:5173–80.
- Kapur A, Harries AD. The double burden of diabetes and tuberculosis - public health implications. *Diabetes Res Clin Pract* 2013;101:10–19.
- Kaufmann E, Spohr C, Battenfeld S et al. BCG vaccination induces robust CD4+ T cell responses to Mycobacterium tuberculosis complex-specific lipopeptides in guinea pigs. *J Immunol* 2016;196:2723–32.
- Kaufmann SH, Cole ST, Mizrahi V et al. Mycobacterium tuberculosis and the host response. *J Exp Med* 2005;201:1693–7.
- Kaushal D, Foreman TW, Gautam US et al. Mucosal vaccination with attenuated Mycobacterium tuberculosis induces strong central memory responses and protects against tuberculosis. *Nat Commun* 2015;6:8533.
- Kaushal D, Mehra S. Faithful experimental models of human infection. *Mycobact Dis* 2012;2:e108.
- Kaushal D, Mehra S, Didier PJ et al. The non-human primate model of tuberculosis. *J Med Primatol* 2012;41:191–201.
- Kaushal D, Schroeder BG, Tyagi S et al. Reduced immunopathology and mortality despite tissue persistence in a Mycobacterium tuberculosis mutant lacking alternative sigma factor, SigH. *Proc Natl Acad Sci USA* 2002;99:8330–5.
- Kelley CL, Collins FM. Growth of a highly virulent strain of Mycobacterium tuberculosis in mice of differing susceptibility to tuberculous challenge. *Tuber Lung Dis* 1999;79:367–70.
- Kesavan AK, Brooks M, Tufariello J et al. Tuberculosis genes expressed during persistence and reactivation in the resistant rabbit model. *Tuberculosis (Edinb)* 2009;89:17–21.
- Khader SA, Bell GK, Pearl JE et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nat Immunol* 2007;8:369–77.
- Khader SA, Pearl JE, Sakamoto K et al. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. *J Immunol* 2005;175:788–95.
- Kimata JT. Stepping toward a macaque model of HIV-1 induced AIDS. *Viruses* 2014;6:3643–51.
- Kitano M, Itoh Y, Kodama M et al. Establishment of a cynomolgus macaque model of influenza B virus infection. *Virology* 2010;407:178–84.
- Kramnik I. Genetic dissection of host resistance to Mycobacterium tuberculosis: the sst1 locus and the Ipr1 gene. *Curr Top Microbiol Immunol* 2008;321:123–48.
- Kramnik I, Beamer G. Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. *Semin Immunopathol* 2016;38:221–37.
- Kramnik I, Demant P, Bloom BB. Susceptibility to tuberculosis as a complex genetic trait: analysis using recombinant congenic strains of mice. *Novartis Found Symp* 1998;217:120–31.
- Kubler A, Luna B, Larsson C et al. Mycobacterium tuberculosis dysregulates MMP/TIMP balance to drive rapid cavitation and unrestrained bacterial proliferation. *J Pathol* 2015;235:431–44.
- Kumar A, Farhana A, Guidry L et al. Redox homeostasis in mycobacteria: the key to tuberculosis control? *Expert Rev Mol Med* 2011;13:e39.
- Kuroda MJ, Sugimoto C, Cai Y et al. High turnover of tissue macrophages contributes to tuberculosis reactivation in

- simian immunodeficiency virus-infected rhesus macaques. *J Infect Dis* 2018;**217**:1865–74.
- Kwan CK, Ernst JD. HIV and tuberculosis: a deadly human syndemic. *Clin Microbiol Rev* 2011;**24**:351–76.
- Lagranderie MR, Balazuc AM, Deriaud E et al. Comparison of immune responses of mice immunized with five different *Mycobacterium bovis* BCG vaccine strains. *Infect Immun* 1996;**64**:1–9.
- Lamichhane G, Tyagi S, Bishai WR. Designer arrays for defined mutant analysis to detect genes essential for survival of *Mycobacterium tuberculosis* in mouse lungs. *Infect Immun* 2005;**73**:2533–40.
- Lamichhane G, Zignol M, Blades NJ et al. A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: application to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2003;**100**:7213–8.
- Langermans JA, Andersen P, van Soolingen D et al. Divergent effect of bacillus Calmette-Guérin (BCG) vaccination on *Mycobacterium tuberculosis* infection in highly related macaque species: implications for primate models in tuberculosis vaccine research. *Proc Natl Acad Sci USA* 2001;**98**:11497–502.
- Lenaerts AJ, Bitting C, Woolhiser L et al. Evaluation of a 2-pyridone, KRQ-10018, against *Mycobacterium tuberculosis* in vitro and in vivo. *Antimicrob Agents Chemother* 2008;**52**:1513–5.
- Lenaerts AJ, Hoff D, Aly S et al. Location of persisting mycobacteria in a guinea pig model of tuberculosis revealed by r207910. *Antimicrob Agents Chemother* 2007;**51**:3338–45.
- Lewinsohn DM, Tydeman IS, Frieder M et al. High resolution radiographic and fine immunologic definition of TB disease progression in the rhesus macaque. *Microbes Infect* 2006;**8**:2587–98.
- Lin PL, Coleman T, Carney JP et al. Radiologic responses in cynomolgous macaques for assessing tuberculosis chemotherapy regimens. *Antimicrob Agents Chemother* 2013;**57**:4237–44.
- Lin PL, Dietrich J, Tan E et al. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection. *J Clin Invest* 2012;**122**:303–14.
- Lin PL, Flynn JL. CD8 T cells and *Mycobacterium tuberculosis* infection. *Semin Immunopathol* 2015;**37**:239–49.
- Lin PL, Ford CB, Coleman MT et al. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat Med* 2014;**20**:75–9.
- Lin PL, Pawar S, Myers A et al. Early events in *Mycobacterium tuberculosis* infection in cynomolgus macaques. *Infect Immun* 2006;**74**:3790–803.
- Lin PL, Rodgers M, Smith L et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. *Infect Immun* 2009;**77**:4631–42.
- Logan RW, Robledo RF, Recla JM et al. High-precision genetic mapping of behavioral traits in the diversity outbred mouse population. *Genes Brain Behav* 2013;**12**:424–37.
- Lombardini ED, Gettayacamin M, Turner GD et al. A review of plasmodium coatneyi-macaque models of severe malaria. *Vet Pathol* 2015;**52**:998–1011.
- Lurie MB, Zappasodi P, Tickner C. On the nature of genetic resistance to tuberculosis in the light of the host-parasite relationships in natively resistant and susceptible rabbits. *Am Rev Tuberc* 1955;**72**:297–329.
- Maiello P, DiFazio RM, Cadena AM et al. Rhesus macaques are more susceptible to progressive tuberculosis than cynomolgus macaques: A quantitative comparison. *Infect Immun* 2017.
- Manabe YC, Dannenberg AM, Jr., Tyagi SK et al. Different strains of *Mycobacterium tuberculosis* cause various spectrums of disease in the rabbit model of tuberculosis. *Infect Immun* 2003;**71**:6004–11.
- Manabe YC, Kesavan AK, Lopez-Molina J et al. The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. *Tuberculosis (Edinb)* 2008;**88**:187–96.
- Manca C, Tsenova L, Barry CE, 3rd et al. *Mycobacterium tuberculosis* CDC1551 induces a more vigorous host response in vivo and in vitro, but is not more virulent than other clinical isolates. *J Immunol* 1999;**162**:6740–6.
- Manganelli R, Dubnau E, Tyagi S et al. Differential expression of 10 sigma factor genes in *Mycobacterium tuberculosis*. *Mol Microbiol* 1999;**31**:715–24.
- Manganelli R, Voskuil MI, Schoolnik GK et al. The *Mycobacterium tuberculosis* ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. *Mol Microbiol* 2001;**41**:423–37.
- Martin CJ, Cadena AM, Leung VW et al. Digitally barcoding *Mycobacterium tuberculosis* reveals in vivo infection dynamics in the macaque model of tuberculosis. *MBio* 2017;**8**:e00312–17.
- Martino M, Hubbard GB, Schlabritz-Loutsevitch N. Tuberculosis (*Mycobacterium tuberculosis*) in a pregnant baboon (*Papio cynocephalus*). *J Med Primatol* 2007;**36**:108–12.
- Mattila JT, Ojo OO, Kepka-Lenhart D et al. Microenvironments in tuberculous granulomas are delineated by distinct populations of macrophage subsets and expression of nitric oxide synthase and arginase isoforms. *J Immunol* 2013;**191**:773–84.
- McDermott JE, Shankaran H, Eisfeld AJ et al. Conserved host response to highly pathogenic avian influenza virus infection in human cell culture, mouse and macaque model systems. *BMC Syst Biol* 2011;**5**:190.
- McGillivray A, Golden NA, Gautam US et al. The *Mycobacterium tuberculosis* Rv2745c plays an important role in responding to redox stress. *PLoS One* 2014;**9**:e93604.
- McHugh KJ, Mandalapu S, Kolls JK et al. A novel outbred mouse model of 2009 pandemic influenza and bacterial co-infection severity. *PLoS One* 2013;**8**:e82865.
- Mehra S, Alvarez X, Didier PJ et al. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by *Mycobacterium tuberculosis*. *J Infect Dis* 2013;**207**:1115–27.
- Mehra S, Foreman TW, Didier PJ et al. The DosR regulon modulates adaptive immunity and is essential for *M. tuberculosis* persistence. *Am J Respir Crit Care Med* 2015;**191**:1185–96.
- Mehra S, Golden NA, Stuckey K et al. The *Mycobacterium tuberculosis* stress response factor SigH is required for bacterial burden as well as immunopathology in primate lungs. *J Infect Dis* 2012;**205**:1203–13.
- Mehra S, Pahar B, Dutta NK et al. Transcriptional reprogramming in nonhuman primate (rhesus macaque) tuberculosis granulomas. *PLoS One* 2010;**5**:e12266.
- Migliori GB, Centis R, D'Ambrosio L et al. Totally drug-resistant and extremely drug-resistant tuberculosis: the same disease? *Clin Infect Dis* 2012;**54**:1379–80.
- Monin L, Griffiths KL, Lam WY et al. Helminth-induced arginase-1 exacerbates lung inflammation and disease severity in tuberculosis. *J Clin Invest* 2015;**125**:4699–713.
- Napier RJ, Norris BA, Swimm A et al. Low doses of imatinib induce myelopoiesis and enhance host anti-microbial immunity. *PLoS Pathog* 2015;**11**:e1004770.
- Napier RJ, Rafi W, Cheruvu M et al. Imatinib-sensitive tyrosine kinases regulate mycobacterial pathogenesis and represent

- therapeutic targets against tuberculosis. *Cell Host Microbe* 2011;**10**:475–85.
- Nedeltchev GG, Raghunand TR, Jassal MS et al. Extrapulmonary dissemination of *Mycobacterium bovis* but not *Mycobacterium tuberculosis* in a bronchoscopic rabbit model of cavitary tuberculosis. *Infect Immun* 2009;**77**:598–603.
- Niazi MK, Dhulekar N, Schmidt D et al. Lung necrosis and neutrophils reflect common pathways of susceptibility to *Mycobacterium tuberculosis* in genetically diverse, immune-competent mice. *Dis Model Mech* 2015;**8**:1141–53.
- North RJ. *Mycobacterium tuberculosis* is strikingly more virulent for mice when given via the respiratory than via the intravenous route. *J Infect Dis* 1995;**172**:1550–3.
- Nueremberger E, Tyagi S, Tasneen R et al. 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**:1522–4.
- Nunes-Alves C, Booty MG, Carpenter SM et al. In search of a new paradigm for protective immunity to TB. *Nat Rev Microbiol* 2014;**12**:289–99.
- Nusbaum RJ, Calderon VE, Huante MB et al. Pulmonary tuberculosis in humanized mice infected with HIV-1. *Sci Rep* 2016;**6**:21522.
- Ordway D, Henao-Tamayo M, Harton M et al. The hypervirulent *Mycobacterium tuberculosis* strain HN878 induces a potent TH1 response followed by rapid down-regulation. *J Immunol* 2007;**179**:522–31.
- Orme IM. The mouse as a useful model of tuberculosis. *Tuberculosis (Edinb)* 2003;**83**:112–5.
- Peretti S, Shaw A, Blanchard J et al. Immunomodulatory effects of HSV-2 infection on immature macaque dendritic cells modify innate and adaptive responses. *Blood* 2005;**106**:1305–13.
- Phares TW, May AD, Genito CJ et al. Rhesus macaque and mouse models for down-selecting circumsporozoite protein based malaria vaccines differ significantly in immunogenicity and functional outcomes. *Malar J* 2017;**16**:115.
- Philipp MT, Doyle LA, Martin DS et al. A rhesus macaque model of *Streptococcus pneumoniae* carriage. *J Med Primatol* 2012;**41**:60–6.
- Phillips BL, Mehra S, Ahsan MH et al. LAG3 expression in active *Mycobacterium tuberculosis* infections. *Am J Pathol* 2015;**185**:820–33.
- Piddington DL, Fang FC, Laessig T et al. Cu,Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. *Infect Immun* 2001;**69**:4980–7.
- Podell BK, Ackart DF, Obregon-Henao A et al. Increased severity of tuberculosis in guinea pigs with type 2 diabetes: a model of diabetes-tuberculosis comorbidity. *Am J Pathol* 2014;**184**:1104–18.
- Podell BK, Ackart DF, Richardson MA et al. A model of type 2 diabetes in the guinea pig using sequential diet-induced glucose intolerance and streptozotocin treatment. *Dis Model Mech* 2017;**10**:151–62.
- Pym AS, Saint-Joanis B, Cole ST. Effect of katG mutations on the virulence of *Mycobacterium tuberculosis* and the implication for transmission in humans. *Infect Immun* 2002;**70**:4955–60.
- Qiu L, Huang D, Chen CY et al. Severe tuberculosis induces unbalanced up-regulation of gene networks and overexpression of IL-22, MIP-1alpha, CCL27, IP-10, CCR4, CCR5, CXCR3, PD1, PDL2, IL-3, IFN-beta, TIM1, and TLR2 but low antigen-specific cellular responses. *J Infect Dis* 2008;**198**:1514–9.
- Ramakrishnan L. The zebrafish guide to tuberculosis immunity and treatment. *Cold Spring Harb Symp Quant Biol* 2013;**78**:179–92.
- Rao V, Fujiwara N, Porcelli SA et al. *Mycobacterium tuberculosis* controls host innate immune activation through cyclopropane modification of a glycolipid effector molecule. *J Exp Med* 2005;**201**:535–43.
- Reiley WW, Calayag MD, Wittmer ST et al. ESAT-6-specific CD4 T cell responses to aerosol *Mycobacterium tuberculosis* infection are initiated in the mediastinal lymph nodes. *Proc Natl Acad Sci USA* 2008;**105**:10961–6.
- Ribi E, Anacker RL, Barclay WR et al. Efficacy of mycobacterial cell walls as a vaccine against airborne tuberculosis in the Rhesus monkey. *J Infect Dis* 1971;**123**:527–38.
- Rifat D, Prideaux B, Savic RM et al. Pharmacokinetics of rifapentine and rifampin in a rabbit model of tuberculosis and correlation with clinical trial data. *Sci Transl Med* 2018;**10**:eaai7786.
- Rincon-Choles H, Abboud HE, Lee S et al. Renal histopathology of a baboon model with type 2 diabetes. *Toxicol Pathol* 2012;**40**:1020–30.
- Rojas-Espinosa O, Dannenberg AM, Jr., Sternberger LA et al. The role of cathepsin D in the pathogenesis of tuberculosis. A histochemical study employing unlabeled antibodies and the peroxidase-antiperoxidase complex. *Am J Pathol* 1974;**74**:1–17.
- Rosenthal IM, Zhang M, Williams KN et al. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. *PLoS Med* 2007;**4**:e344.
- Rowley WR, Bezold C, Arikan Y et al. Diabetes 2030: insights from yesterday, today, and future trends. *Popul Health Manag* 2017;**20**:6–12.
- Sassetti CM, Boyd DH, Rubin EJ. Comprehensive identification of conditionally essential genes in mycobacteria. *Proc Natl Acad Sci USA* 2001;**98**:12712–7.
- Sassetti CM, Rubin EJ. Genetic requirements for mycobacterial survival during infection. *Proc Natl Acad Sci USA* 2003;**100**:12989–94.
- Sestak K, Conroy L, Aye PP et al. Improved xenobiotic metabolism and reduced susceptibility to cancer in gluten-sensitive macaques upon introduction of a gluten-free diet. *PLoS One* 2011;**6**:e18648.
- Sharpe S, White A, Gleeson F et al. Ultra low dose aerosol challenge with *Mycobacterium tuberculosis* leads to divergent outcomes in rhesus and cynomolgus macaques. *Tuberculosis (Edinb)* 2016;**96**:1–12.
- Sharpe SA, Eschelbach E, Basaraba RJ et al. Determination of lesion volume by MRI and stereology in a macaque model of tuberculosis. *Tuberculosis (Edinb)* 2009;**89**:405–16.
- Sharpe SA, McShane H, Dennis MJ et al. Establishment of an aerosol challenge model of tuberculosis in rhesus macaques and an evaluation of endpoints for vaccine testing. *Clin Vaccine Immunol* 2010;**17**:1170–82.
- Singhal A, Aliouat el M, Hervé M et al. Experimental tuberculosis in the Wistar rat: a model for protective immunity and control of infection. *PLoS One* 2011;**6**:e18632.
- Singhal A, Jie L, Kumar P et al. Metformin as adjunct antituberculosis therapy. *Sci Transl Med* 2014;**6**:263ra159.
- Slight SR, Rangel-Moreno J, Gopal R et al. CXCR5(+) T helper cells mediate protective immunity against tuberculosis. *J Clin Invest* 2013;**123**:712–26.
- Smith CM, Proulx MK, Olive AJ et al. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. *MBio* 2016;**7**:e01516–16.

- Society AT. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR Recomm Rep* 2000;**49**(RR-6):1–51.
- Stallings CL, Glickman MS. Is *Mycobacterium tuberculosis* stressed out? A critical assessment of the genetic evidence. *Microbes Infect* 2010;**12**:1091–101.
- Subbian S, Bandyopadhyay N, Tsenova L et al. Early innate immunity determines outcome of *Mycobacterium tuberculosis* pulmonary infection in rabbits. *Cell Commun Signal* 2013a;**11**:60.
- Subbian S, O'Brien P, Kushner NL et al. Molecular immunologic correlates of spontaneous latency in a rabbit model of pulmonary tuberculosis. *Cell Commun Signal* 2013b;**11**:16.
- Subbian S, Tsenova L, Holloway J et al. Adjunctive phosphodiesterase-4 inhibitor therapy improves antibiotic response to pulmonary tuberculosis in a rabbit model. *EBioMedicine* 2016;**4**:104–14.
- Subbian S, Tsenova L, O'Brien P et al. Phosphodiesterase-4 inhibition alters gene expression and improves isoniazid-mediated clearance of *Mycobacterium tuberculosis* in rabbit lungs. *PLoS Pathog* 2011a;**7**:e1002262.
- Subbian S, Tsenova L, O'Brien P et al. Spontaneous latency in a rabbit model of pulmonary tuberculosis. *Am J Pathol* 2012;**181**:1711–24.
- Subbian S, Tsenova L, Yang G et al. Chronic pulmonary cavitory tuberculosis in rabbits: a failed host immune response. *Open Biol* 2011b;**1**:110016.
- Sugawara I, Mizuno S. Higher susceptibility of type 1 diabetic rats to *Mycobacterium tuberculosis* infection. *Tohoku J Exp Med* 2008;**216**:363–70.
- Sugawara I, Yamada H, Mizuno S. Pathological and immunological profiles of rat tuberculosis. *Int J Exp Pathol* 2004a;**85**:125–34.
- Sugawara I, Yamada H, Mizuno S. Pulmonary tuberculosis in spontaneously diabetic goto kakizaki rats. *Tohoku J Exp Med* 2004b;**204**:135–45.
- Svenson KL, Gatti DM, Valdar W et al. High-resolution genetic mapping using the mouse diversity outbred population. *Genetics* 2012;**190**:437–47.
- Swaim LE, Connolly LE, Volkman HE et al. *Mycobacterium marinum* infection of adult zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. *Infect Immun* 2006;**74**:6108–17.
- Tobin DM, Vary JC, Jr., Ray JP et al. The *lta4h* locus modulates susceptibility to mycobacterial infection in zebrafish and humans. *Cell* 2010;**140**:717–30.
- Tree JA, Elmore MJ, Javed S et al. Development of a guinea pig immune response-related microarray and its use to define the host response following *Mycobacterium bovis* BCG vaccination. *Infect Immun* 2006;**74**:1436–41.
- Tsenova L, Ellison E, Harbacheuski R et al. Virulence of selected *Mycobacterium tuberculosis* clinical isolates in the rabbit model of meningitis is dependent on phenolic glycolipid produced by the bacilli. *J Infect Dis* 2005;**192**:98–106.
- Tsenova L, O'Brien P, Holloway J et al. Etanercept exacerbates inflammation and pathology in a rabbit model of active pulmonary tuberculosis. *J Interferon Cytokine Res* 2014;**34**:716–26.
- Turner OC, Basaraba RJ, Orme IM. Immunopathogenesis of pulmonary granulomas in the guinea pig after infection with *Mycobacterium tuberculosis*. *Infect Immun* 2003;**71**:864–71.
- Vandal OH, Nathan CF, Ehrt S. Acid resistance in *Mycobacterium tuberculosis*. *J Bacteriol* 2009;**191**:4714–21.
- Vandal OH, Pierini LM, Schnappinger D et al. A membrane protein preserves intrabacterial pH in intraphagosomal *Mycobacterium tuberculosis*. *Nat Med* 2008;**14**:849–54.
- Varela C, Rittmann D, Singh A et al. *MmpL* genes are associated with mycolic acid metabolism in mycobacteria and corynebacteria. *Chem Biol* 2012;**19**:498–506.
- Veazey RS, DeMaria M, Chalifoux LV et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 1998;**280**:427–31.
- Verreck FA, Vervenne RA, Kondova I et al. MVA.85A boosting of BCG and an attenuated, *phoP* deficient *M. tuberculosis* vaccine both show protective efficacy against tuberculosis in rhesus macaques. *PLoS One* 2009;**4**:e5264.
- Verreck FAW, Tchilian EZ, Vervenne RAW et al. Variable BCG efficacy in rhesus populations: pulmonary BCG provides protection where standard intra-dermal vaccination fails. *Tuberculosis (Edinb)* 2017;**104**:46–57.
- Via LE, England K, Weiner DM et al. A sterilizing tuberculosis treatment regimen is associated with faster clearance of bacteria in cavitory lesions in marmosets. *Antimicrob Agents Chemother* 2015;**59**:4181–9.
- Via LE, Schimel D, Weiner DM et al. Infection dynamics and response to chemotherapy in a rabbit model of tuberculosis using [(1)(8)F]2-fluoro-deoxy-D-glucose positron emission tomography and computed tomography. *Antimicrob Agents Chemother* 2012;**56**:4391–402.
- Via LE, Weiner DM, Schimel D et al. Differential virulence and disease progression following *Mycobacterium tuberculosis* complex infection of the common marmoset (*Callithrix jacchus*). *Infect Immun* 2013;**81**:2909–19.
- Volkman HE, Clay H, Beery D et al. Tuberculous granuloma formation is enhanced by a mycobacterium virulence determinant. *PLoS Biol* 2004;**2**:e367.
- Volkman HE, Pozos TC, Zheng J et al. Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. *Science* 2010;**327**:466–9.
- Walsh GP, Tan EV, dela Cruz EC et al. The Philippine cynomolgus monkey (*Macaca fascicularis*) provides a new nonhuman primate model of tuberculosis that resembles human disease. *Nat Med* 1996;**2**:430–6.
- Weed M, Adams RJ, Hienz RD et al. SIV/macaque model of HIV infection in cocaine users: minimal effects of cocaine on behavior, virus replication, and CNS inflammation. *J Neuroimmune Pharmacol* 2012;**7**:401–11.
- Weiss LA, Stallings CL. Essential roles for *Mycobacterium tuberculosis* Rel beyond the production of (p)ppGpp. *J Bacteriol* 2013;**195**:5629–38.
- Weyand NJ, Wertheimer AM, Hobbs TR et al. *Neisseria* infection of rhesus macaques as a model to study colonization, transmission, persistence, and horizontal gene transfer. *Proc Natl Acad Sci USA* 2013;**110**:3059–64.
- White AG, Maiello P, Coleman MT et al. Analysis of 18FDG PET/CT imaging as a tool for studying mycobacterium tuberculosis infection and treatment in non-human primates. *J Vis Exp* 2017, DOI: 10.3791/56375.
- WHO. *Global Tuberculosis Report 2017*. 2017, Geneva: World Health Organization.
- Winter JM, Gildea DE, Andreas JP et al. Mapping complex traits in a diversity outbred f1 mouse population identifies germline modifiers of metastasis in human prostate cancer. *Cell Syst* 2017;**4**:31–45 e6.
- Winthrop KL, McNelley E, Kendall B et al. Pulmonary nontuberculous mycobacterial disease prevalence and clinical features:

- an emerging public health disease. *Am J Respir Crit Care Med* 2010;**182**:977–82.
- Wolf AJ, Desvignes L, Linas B et al. Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *J Exp Med* 2008;**205**:105–15.
- Wong EA, Joslyn L, Grant NL et al. Low levels of T cell exhaustion in tuberculous lung granulomas. *Infect Immun* 2018;**86**:e00426–18.
- Zak DE, Penn-Nicholson A, Scriba TJ et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet* 2016;**387**:2312–22.