Murine models of susceptibility to tuberculosis

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Summary

Approximately one third of the world’s population is infected with Mycobacterium tuberculosis, yet each year a small proportion of those individuals progress to an active disease state. Early identification and treatment of such individuals is essential to reduce transmission; however, genetic and immunological correlates of disease progression have not been well established in man. The murine model has been a central tool for the elucidation of protective immune mechanisms that are essential for controlling M. tuberculosis infection. Additionally, the study of inbred mice has revealed significant divergence in the susceptibility and disease progression of individual mouse strains to an infection with M. tuberculosis. The continued study of genetically disparate mouse strains has the potential to identify immune mechanisms that correlate with increasing susceptibility to tuberculosis. These mechanisms will be highly applicable to studies in man and assist in the early detection of individuals that are more vulnerable to the development of reactivation tuberculosis.

Key words: tuberculosis • reactivation • murine • lung
INTRODUCTION

It has been estimated that one third of the human population harbors Mycobacterium tuberculosis (M. tuberculosis)\(^24, 48\), the human pathogen responsible for causing tuberculosis (TB). The majority of individuals infected with this pathogen will acquire long-lasting immunity that effectively controls the infection within the lung. In contrast, between 5–10% of infected individuals will develop significant pulmonary disease that will progress to an active disease state where M. tuberculosis can be efficiently transmitted to other individuals\(^29\). There are several known risk factors that positively correlate with progression to clinically significant disease, including malnutrition, concurrent infection with human immunodeficiency virus, and increasing age\(^16, 28, 31, 77\). The majority of individuals who develop pulmonary TB do not, however, demonstrate any of those predisposing conditions; subtle differences in host genetics and immunity likely contribute to the variable course of infection. The immunological and genetic determinants that separate individuals that control an infection (latency) from individuals that will develop fulminant TB (reactivation) have not been fully elucidated.

Numerous human twin and population studies have shown that a multifactoral genetic influence is apparent in pulmonary M. tuberculosis infections. Linkage analyses have identified several chromosomal regions and genetic loci that are associated with increased susceptibility or resistance to TB. These include chromosome 15, the X chromosome, as well as genes encoding several cytokines and cytokine receptors, major histocompatibility proteins, iron transporter proteins, and the vitamin D receptor\(^4–6, 34, 86\). Genetic studies in mice have discovered at least 5 different loci that are associated with increased susceptibility or resistance to TB, and more recent genetic studies indicate that the expression of disease severity is under multigenic control\(^49, 50, 60, 68, 74\). Thus, in human and murine studies, resistance or susceptibility to TB cannot be explained by the expression of a single gene. The final outcome (control of infection vs fulminating disease) results from complicated interactions between the host immune response and M. tuberculosis organisms. Identification, clarification, and manipulation of the immunological parameters that lead to increased susceptibility to M. tuberculosis infection would provide crucial information for understanding disease pathogenesis and the requirements for host immunity.

Correlates of protective immunity can be determined using animal models of infection. The murine model is particularly attractive due to the numerous resources available to the research community and the wealth of information already published regarding susceptibility to TB. Abundant studies using C57BL/6 and BALB/c inbred strains of mice, including gene-disrupted mice on these backgrounds, have identified several key protective cells and cytokines that are essential for controlling an infection with M. tuberculosis\(^22, 23, 30\). The immune response that is generated during infection with M. tuberculosis is primarily characterized by a T-helper 1 (TH1) cytokine profile, with CD4 T cell-derived interferon (IFN)-\(^\gamma\), and the secretion of interleukin (IL)-12 and tumor necrosis factor (TNF) by macrophages\(^39\). Within the lung, the primary host cell for M. tuberculosis is the alveolar macrophage; however, acid-fast bacilli can be readily found in resident or infiltrating tissue macrophages\(^71\). Following infection, macrophages secrete inflammatory mediators such as TNF, IL-1, and IL-6 and also produce chemokines which facilitate the recruitment of antigen-specific T cells to the site of infection\(^29\). Infected dendritic cells traffic to the draining lymph nodes and initiate acquired immunity\(^7\). The generation of antigen-specific TH1 cells that can migrate into the infected lung further activates macrophage microbicidal/static mechanisms, increasing the production of reactive oxygen and nitrogen intermediates\(^15, 25\) and resulting in cessation of mycobacterial growth, but not necessarily sterilization of infection, within the lung. The requirement for TH1 immunity has been verified by many studies which demonstrate that IFN-\(^\gamma\), IL-12, and IL-18 are essential for the control of an infection with M. tuberculosis\(^20, 22, 47\). In man, defects in specific TH1 signaling pathways lead to increased susceptibility to mycobacterial infection\(^3\), further confirming that TH1 immunity is necessary for controlling an infection with M. tuberculosis. Additionally, patients administered TNF blockers are highly susceptible to developing active TB, most likely due to the reactivation of a previously latent infection\(^45\). The generation of TH1-mediated immunity is clearly essential, but may not be sufficient, for the control of M. tuberculosis infection, as a proportion of humans, and all mouse strains tested, remain susceptible to infection with M. tuberculosis despite the generation of a robust TH1 immune response. It is evident that those immune mechanisms associated with the control of an infection with M. tuberculosis and, more significantly, those mechanisms associated with increased susceptibility require further investigation.

The majority of studies that have used the murine model to investigate protective immunity to infection with M. tuberculosis have used two common inbred mouse strains, C57BL/6 and BALB/c. These strains...
have been fundamental in our understanding that TH1 cell-mediated immune responses correlate with the long-term control of an infection with *M. tuberculosis*. Indeed, these two particular mouse strains control an infection with *M. tuberculosis* for an extended period of time and do not show any signs of reactivation of an infection until well into their second year of life. This late reactivation has been attributed to alterations that take place in the immune system during old age that permit the increasing bacterial burden within the lungs. It is well documented that with increasing age, the function of CD4 T cells declines, with delayed or decreased production of IL-2 and an inability to optimally respond to antigen. More specifically, in old mice infected with *M. tuberculosis* there is delayed recruitment or expansion of antigen-specific CD4 T cells into the lungs, leading to ineffective control of infection. Such defects in CD4 T cell immunity are thought to contribute to the increased susceptibility of elderly people to develop primary or reactivation TB. Unfortunately, reactivation of *M. tuberculosis* infection late in life, as a consequence of an aging immune system, is not necessarily indicative of the human population, where the majority of TB cases are diagnosed at middle-age.

The identification of alternative mouse strains that model the course of infection will lead to the discovery of immune responses that correlate with susceptibility to, and reactivation of, an infection with *M. tuberculosis*.

**MODELS OF REACTIVATION TB**

Use of the murine model to understand the transition from latency to reactivation of an infection with *M. tuberculosis* is complicated by the fact that the mouse is an experimental host for *M. tuberculosis*. Disease progression in the murine models of pulmonary TB may not reflect the clinically defined stages of latent and reactivation *M. tuberculosis* infection in man. In particular, mice harbor a relatively high bacterial load within the lungs in a chronic state of infection for months. In contrast, latent *M. tuberculosis* infection in man is associated with low numbers of bacteria that likely persist for months to years in a non-replicating state. When considering data obtained from animal models it is necessary to understand and critically evaluate the limitations of each system. Experiments must be designed to rationally model the particular disease state of interest in man. Using this approach, the murine model has increased our understanding of the immunological requirements for the containment of *M. tuberculosis* infection. Importantly, these observations, such as the requirement for TNF, have been applicable to studies in man.

The mouse model of TB has been modified to account for the substantially elevated bacterial load normally found within the lungs. This has been achieved with the Cornell model, or modifications of it, in which a short course of anti-tuberculous drugs is administered to mice following an infection with *M. tuberculosis*. In doing so, the bacterial load within the lungs of mice becomes undetectable by standard plating techniques. The small number of remaining bacteria within the lungs may depict latent infection in man; however, the potential for mycobacterial genetic or metabolic modifications secondary to the drug regimen has not been fully addressed and has led to reservations about the suitability of this model. What is known, however, is that this model of latency can be reactivated upon administration of immunosuppressive agents. Using the Cornell model, or variations of this model, it has been shown that reactivation of *M. tuberculosis* can be induced by glucocorticoid treatment, anti-CD8, nitric oxide inhibitors, and anti-TNF, thereby demonstrating the requirement for several immunological mediators in the control of *M. tuberculosis*. More significantly, in the absence of immunosuppressive treatment, *M. tuberculosis* eventually reactivates within the lungs of all immunocompetent mice, again confirming that host immunity in the mouse model remains ineffective in controlling latent TB. Natural reactivation of a latent *M. tuberculosis* infection, in the absence of immunomodulatory experimental intervention, provides a good model for reactivation; however, the period between drug treatment and reactivation of infection can be highly sporadic in C57BL/6 mice, making this model challenging to study.

An alternative approach to the Cornell model of drug-induced latent infection is to examine the natural progression of *M. tuberculosis* infection in the mouse model. This approach assumes that, despite the elevated bacterial load within the lungs of infected mice, the chronicity of *M. tuberculosis* infection and stable bacterial burden in the mouse is comparable to a state of latency in man. In other words, despite the difference in the number of bacteria in the lungs of humans and in mice, the host’s immune response adequately controls bacterial proliferation for extended periods of time. Eventually, in murine models of TB, bacterial proliferation resumes and all mice die secondary to overwhelming pulmonary disease. Despite the initially high (but controlled) bacterial load in the lungs of infected mice, this form of natural reactivation is an attractive model of disease progression and susceptibility because the customary course of control and reactivation of infection occurs without any intervention. Additionally, as the study of long-term chronic TB continues in the mouse...
model, the ability to accurately predict the time-frame of reactivation has improved, making this a more appealing system than the Cornell model.

While we are only just beginning to understand these natural reactivation models and their applications to studies in man, data obtained from the mouse model has supported many of the immunological findings in man. An understanding of the subtle shifts in the immune response during long-term chronic infection that eventually result in reactivation will provide insight regarding the immune responses necessary to maintain a long-term infection with *M. tuberculosis* in man. The observation that different mouse strains reactivate infection with *M. tuberculosis* at highly predictable times post-infection has provided the research community with tools to investigate how individual populations (or mouse strains) differentially respond to and control infection with this pathogen. Ultimately, this information should be beneficial to studies of a highly heterogeneous human population.

**MOUSE STRAINS CAN BE DIVIDED INTO CLUSTERS OF SUSCEPTIBLE OR RESISTANT STRAINS ACCORDING TO THEIR ABILITY TO SURVIVE AN INFECTION WITH *M. TUBERCULOSIS***

One of the earliest studies investigating the susceptibility of different mouse strains to infection with *M. tuberculosis* was performed by Pierce et al. in 1947. The results indicated substantial variation in the susceptibility of 22 different mouse strains to infection with virulent *M. tuberculosis* H37Rv. These studies were further substantiated by Medina and North, who ranked specific inbred mouse strains according to their survival following either intravenous or aerosol infection with *M. tuberculosis* H37Rv. Although survival studies revealed a broad spectrum of susceptibility to *M. tuberculosis* infection, mouse strains could be easily classified into two major categories: “susceptible” or “resistant.”

Those mouse strains that survived an intravenous infection with *M. tuberculosis* for more than 300 days were termed “resistant” to infection, and included the commonly used C57BL/6 and BALB/c mouse strains. In contrast, mouse strains that succumbed to infection more rapidly were termed “susceptible.” Susceptible mouse strains included the CBA/J and DBA/2 strains, which survived an intravenous infection with *M. tuberculosis* for 150 days or less. Similar survival patterns in these different mouse strains were obtained using the low-dose aerosol model of infection. Using intravenous or aerosol infection, recent studies have also classified the A/J, 42, 50 and SWR mouse strains as highly susceptible to infection with *M. tuberculosis* Erdman. The fundamental difference between studies that used the intravenous route of infection is the period of time between the establishment of infection and death due to pulmonary disease, with mice infected by the intravenous route succumbing to infection much sooner than those with aerosol infection. Despite the differences in survival curves, the pattern of increased susceptibility or resistance remained identical for all mouse strains tested. In a similar manner, the pattern of susceptibility or resistance to infection is preserved independent of the strain of *M. tuberculosis* used. Studies have been performed using Erdman, H37Rv, and clinical isolates, including multi-drug-resistant strains. Despite moderate changes in virulence, bacterial load, and granuloma formation, no impact on the pattern of susceptibility with regard to survival time has been observed.

Survival analyses reveal a spectrum of susceptibility patterns between genetically different mouse strains and have identified several mouse strains with increased susceptibility to infection with *M. tuberculosis* compared with the highly resistant common laboratory C57BL/6 or BALB/c mouse strains. These susceptible mouse strains may provide more accurate models for the course of reactivation TB in man. Continued investigation of the immune mechanisms that influence disease progression in different strains of mice will lead to significant advancements in understanding the widely varied susceptibilities to infection with *M. tuberculosis* observed in the heterogeneous human population.

**SUSCEPTIBLE MOUSE STRAINS HAVE INCREASED BACTERIAL LOAD WITHIN THE LUNGS***

Survival studies of different mouse strains provide a general overview of susceptibility to infection with *M. tuberculosis*. To understand the mechanisms of immunity that contribute to extended protection, or that compromise the control of *M. tuberculosis* within the lung, it is necessary to identify, characterize, and understand the specific immunologic events within the lungs of these different mouse strains. For example, short survival time may simply reflect the failure of the host to generate an essential component of protective immunity (such as IFN-γ or TNF, as described above), thus compromising the control of *M. tuberculosis* within the lung. Alternatively, reduced survival time could be a consequence of subtle changes within the lung, such as an increased production of immunosuppressive mediators or, alternatively, an increased production of inflammatory mediators that may drive tissue damage and premature death. In all mice tested, a common theme has
been reported: early death from infection with *M. tuberculosis* is highly associated with increased pulmonary tissue damage. What is not clear, however, is whether the changes within the lung are a direct consequence of the increasing bacterial load or whether damage to pulmonary parenchyma occurs secondary to an inappropriate immune response.

Analysis of the bacterial load within the lungs of several mouse strains during infection with *M. tuberculosis* has revealed a distinct pattern of bacterial growth, leading to the advanced classification of mouse strains into two distinct categories: reactivation resistant and reactivation susceptible. Following an intravenous infection with *M. tuberculosis* Erdman or H37Rv, several of the mouse strains, such as C3H and I/St, failed to limit bacterial growth within the lungs and progressed rapidly to overwhelming disease. The rapid decline of these two mouse strains following an infection with *M. tuberculosis* suggests that some mouse strains are highly susceptible to infection. It is likely, however, that the intravenous administration of *M. tuberculosis* led to the exceptionally short survival times of these particular strains. Another similarly susceptible strain, A/J, can survive much longer when infected via the aerosol rather than intravenous route. Indeed, analysis of I/St mice using the Cornell model of infection has demonstrated that, despite the ability to substantially extend survival, I/St mice remain proportionally more susceptible to reactivation of an infection with *M. tuberculosis* H37Rv than the relatively resistant C57BL/6 mouse strain. These differences in route of infection likely reflect the increased inflammatory response generated when large numbers of mycobacteria are systemically delivered. A direct comparison between high- and low-dose infection with *M. tuberculosis* has demonstrated that an increase in the infectious dose is highly associated with a more rapid disease progression in the I/St and A/J mouse strains.

Several mouse strains could survive an infection with *M. tuberculosis* for considerably longer than those identified above, which was particularly apparent following an aerosol infection. Increasing survival time was highly associated with the ability to halt the exponential growth of *M. tuberculosis* and maintain a stable bacterial load within the lung. The capacity to limit bacterial growth within the lungs did not, however, always translate to long-term survival, and mouse strains could be divided based on their capacity to maintain a chronic infection with *M. tuberculosis*. As previously described, the C57BL/6, BALB/c, and related strains could maintain a prolonged, stable infection with *M. tuberculosis* Erdman or H37Rv for approximately 300 days (Fig. 1). These mice were subsequently termed “reactivation resistant”, as they expressed long-term maintenance of the bacterial load within the lungs. In contrast, other mouse strains (CBA/J, DBA/2) expressed an intermediate phenotype. Calculation of the bacterial load within the lungs of CBA/J and DBA/2 strains of mice revealed a distinct pattern of infection; the growth of *M. tuberculosis* was initially controlled within the lungs and a chronic infection was established. The containment phase of infection was, however, substantially abridged, lasting approximately 150–180 days, and bacterial growth within the lungs eventually resumed (Fig. 1). The characteristic disease progression of the CBA/J and DBA/2 mouse strains has since led to their advanced classification as “reactivation susceptible”.

The capacity of certain mouse strains to initially control and contain an infection with *M. tuberculosis* for a period of time, followed by increasing bacterial load within the lungs, serves as a model of increased susceptibility to TB in man. Despite the limitations of the murine model, in that true latency is not achieved, the immunological response patterns as the disease progresses from a stable chronic infection to one that facilitates the growth of *M. tuberculosis* in the lung can represent reactivation of infection in man. Indeed, several immune parameters that have
been identified in the murine model of reactivation have parallels with individuals who have active TB, as will be discussed in this review. Insight into the mechanisms that lead to the increasing bacterial load within the lungs of reactivation-susceptible mouse strains will lead to a fundamental understanding of why and how individuals that appear to have a latent infection with *M. tuberculosis* can subsequently reactivate.

**Pulmonary lesions are different in reactivation-resistant and -susceptible mouse strains**

One of the fundamental differences between mouse strains that are reactivation resistant or reactivation susceptible is the composition and organization of the pulmonary granulomas. Following an aerosol or intravenous infection with *M. tuberculosis*, reactivation-resistant and -susceptible strains of mice develop a variety of pulmonary microscopic lesions, and these microscopic lesions form predictable patterns that positively correlate with increased susceptibility to *M. tuberculosis* infection and decreased survival. Within the first few weeks following an aerosol infection with *M. tuberculosis*, microscopic lung lesions developed as random, small foci of neutrophils and scattered macrophages, with few perivascular and peribronchiolar lymphocytes. As infection progressed, the numbers of neutrophils declined while macrophages formed more organized sheets and clusters in alveolar sacs and ducts, as well as within the interstitial spaces of alveolar septae. Occasionally, epithelioid macrophages and multinucleated giant cells were noted. Between 3 and 5 weeks post-infection, the lymphocytes began to accumulate within the lungs in substantial numbers, forming halos partially encircling the clusters of macrophages.

As disease progressed beyond the first 6–8 weeks, the number of lymphocytes continued to increase, becoming a dominant cell type within the microscop ic lesions of *M. tuberculosis*-infected reactivation-resistant mouse strains (C57BL/6 and BALB/c). Lymphocytes formed tight, compact, peripheral layers that surrounded clusters of activated, epithelioid, and foamy macrophages. Masson’s trichrome staining of lung tissue from C57BL/6 mice showed progressive deposition of collagenous fibrous connective tissue (fibrosis) within alveolar septae. During the late stages of infection the granulomas enlarged and coalesced, as cells within older areas became disorganized and cholesterol clefts formed. Only in the very late stages of infection (when bacterial numbers are increasing) was necrosis evident, typically occurring as individual cell death and very small foci.

Granuloma formation in reactivation-resistant mouse strains resulted in highly organized aggregates consisting of activated macrophages incompletely rimmed by dense layers of lymphocytes. The microscopic organization of TB granulomas strongly suggests the interface between innate and adaptive immunity critically functions to contain *M. tuberculosis* within discrete areas of the lungs.

In contrast, the microscopic lesions associated with disease progression in reactivation-susceptible mouse strains (such as CBA/J and DBA/2) were substantially different. In these mouse strains, disease progression was associated with the development of multiple large granulomas that were dominated by large numbers of loosely organized, activated (“foamy”) macrophages. Small circular foci of lymphocytes were apparent, but appeared primarily in peribronchietalar and perivascular location. Small numbers of lymphocytes were scattered throughout the macrophage-dominated lesions, yet failed to encircle the sheets and clusters of macrophages. During the end-stages of disease, the macrophage-dominated lesions continued to enlarge and coalesce, and numerous cholesterol clefts, small infiltrations of neutrophils, and larger foci of necrotic cellular debris formed within the center. Additionally, individual macrophages containing intracytoplasmic acid-fast bacilli were randomly scattered throughout the lungs, suggesting that inappropriate granuloma formation and maintenance allowed infected macrophages to disseminate throughout the lungs in reactivation-susceptible mouse strains. In other words, a crucial function of the granuloma is to confine the infection to discrete foci within the lungs and to prevent infected macrophages from migrating and spreading the infection. In contrast to reactivation-resistant mouse strains, disease progression in reactivation-susceptible strains was associated with the generation of large macrophage-dominated lesions and disorganized and unfocused lymphocyte populations that were primarily observed encircling blood vessels and airways. The distinct absence of lymphocyte trafficking and organization suggests that the focusing of lymphocytes into tight aggregates and the containment of infected macrophages within a granuloma are central for the long-term control of an infection with *M. tuberculosis*.

Variation in susceptibility to *M. tuberculosis* infection amongst different mouse strains can also be evident in the formation of compact granulomas. For example, although SWR mice are incapable of establishing a state of chronic infection, they developed lung lesions similar to the reactivation-susceptible DBA/2 and CBA/J mouse strains. Where these strains differ, however, is in the rapidity and severity that the
lesions developed. Within 30 days of infection, the lungs of SWR mice become dominated by a severe, coalescing to diffuse granulomatous pneumonia containing large numbers of intra-alveolar and interstitial foamy and epithelioid macrophages and few lymphocytes. By day 60 post-infection, increased numbers of lymphocytes could be isolated from the lungs of the highly susceptible SWR strain; however, upon microscopic examination, the macrophage infiltrate still predominated. At the end stages of disease, lesions continued to enlarge and coalesce, resulting in lobar consolidation with scattered foci of granulomas continuing to enlarge and coalesce, resulting in lobar consolidation with scattered foci of granulomas. Again, susceptibility to infection with *M. tuberculosis* was highly associated with a deficient lymphocytic response and the development of macrophage-dominated lung lesions.

In contrast to aerosol infection with *M. tuberculosis*, intravenous infection produces an immediate systemic (multi-organ) infection, primarily with large numbers of bacteria localizing to the spleen and liver. Within a short amount of time, mycobacteria seed and proliferate in the lung. Microscopically, the spleens and livers of resistant and susceptible mice were not qualitatively different over time; however, microscopic examination of lung tissue obtained at the same time points post-infection showed patterns identical to the aerosol infections. The reactivation-resistant mouse strains formed compact granulomas composed primarily of lymphocytes, activated, epithelioid, and foamy macrophages with few acid-fast bacilli that filled alveolar sacs and expanded the interstitium of alveolar septae. The lungs of reactivation-susceptible mice contained larger lesions composed of unidentified degenerating and necrotic mononuclear cells, increased numbers of acid-fast bacilli and extensive neutrophilic infiltrates. Although the disease progressed more slowly in reactivation-resistant mice, pulmonary lesions continued to increase in size until overwhelming inflammation and pulmonary insufficiency resulted in death in both resistant and susceptible strains. As defined following aerosol infection, the central difference between the pulmonary lesions of reactivation-resistant and susceptible mice was the lack of lymphocyte infiltration into the lung, indicating again that increased susceptibility to reactivation of *M. tuberculosis* infection likely results from a failure to develop and focus appropriate acquired immunity within the lungs.

**REACTIVATION-SUSCEPTIBLE MICE HAVE FEWER IFN-γ⁺ CD4 T CELLS WITHIN THE LUNGS**

In the extensively studied C57BL/6 mouse strain, immunohistochemical labeling has shown that the compact pulmonary granulomas characteristic of this strain were dominated by CD4 T cells. Additionally, CD4 T cells penetrated the granulomas, and their central location likely reflected the dominant role that CD4 T cells play in controlling *M. tuberculosis* infection. CD8 T cells have also been identified within the lungs of C57BL/6 mice. This cell population, however, was found in a peripheral location to the granuloma. It has been hypothesized that in this position, cytotoxic CD8 T cells detect and eradicate *M. tuberculosis*-infected macrophages that leave the granuloma, thus limiting the spread of the organism to seed other sites. In addition to the numerous T cells found within the lungs of C57BL/6 mice, foci of B cells were also observed. The relevance of this observation is currently unknown, as the absence of B cells appears to have no significant influence on the course of infection with *M. tuberculosis* in C57BL/6 mice. The location of specific lymphocyte subsets within the lungs of reactivation-susceptible mouse strains using immunohistochemical techniques has not been addressed and it is currently unclear where specific lymphocyte subsets are localized within the lung. Subtle changes in the spatial T cell organization within the granulomas of reactivation-susceptible mouse strains could contribute to the increasing susceptibility of the CBA/J and DBA/2 mouse strains to infection with *M. tuberculosis*.

The ability to control and contain an infection with *M. tuberculosis* is highly associated with the capacity of antigen-specific CD4 T cells to secrete IFN-γ locally within the lungs. In response to infection with *M. tuberculosis* Erdman, reactivation-resistant C57BL/6 mice generated a vigorous IFN-γ-producing CD4 T cell population within the lungs that expanded over the first 30 days of infection and stabilized at a relatively high level throughout the entire infection period, consistent with the ability of this mouse strain to control the growth of *M. tuberculosis* within the lung. Culture of cells from the lungs of C57BL/6 mice with the highly immunogenic *M. tuberculosis* culture filtrate proteins revealed that, in response to infection, C57BL/6 mice generated local antigen-specific CD4 T cells that were capable of producing IFN-γ. C57BL/6 mice were, therefore, capable of generating a robust antigen-specific CD4 T cell population within the lungs that could be located around and within the lung granuloma.

In contrast to C57BL/6 mice, CBA/J mice were unable to efficiently expand the CD4 T cell pool, resulting in a significant deficiency of CD4 T cells within the lungs that persisted for at least 120 days post-infection. Similarly, the culture of lung cells from CBA/J mice with culture filtrate proteins from
M. tuberculosis Erdman resulted in significantly less IFN-γ production than seen in C57BL/6 mice, indicating that the generation of antigen-specific immunity was impaired within the lungs of reactivation-susceptible mice. Despite this, sufficient immunity was generated to initially limit the growth of M. tuberculosis within the lungs in a similar manner to C57BL/6 mice, suggesting that the capacity to generate acquired immunity was not completely impaired in reactivation-susceptible mouse strains.

Interestingly, the absence of a robust antigen-specific IFN-γ immune response within the lungs is common to several of the susceptible mouse strains. The susceptible C3H, SWR, and I/St mouse strains are all reported to have deficiencies in the generation of antigen-specific responses, or the production of IFN-γ. Correspondingly, IFN-γ mRNA was also reduced in the lungs of the reactivation-susceptible DBA/2 mouse strain, confirming that, along with the CBA/J mouse strain, a deficiency in IFN-γ production was representative of the reactivation-susceptible cluster of mouse strains. Given that a TH1-mediated CD4 T cell response is critical for the control of an infection with M. tuberculosis, it is highly likely that a suboptimal CD4 T cell response corresponds to an increased susceptibility to infection with M. tuberculosis. Studies that compare antigen-specific IFN-γ production between different reactivation-susceptible mouse strains have not yet been performed; however, there may be a threshold of reactivity required to limit the growth of M. tuberculosis within the lungs that can explain the variability of these mouse strains to succumb to infection with M. tuberculosis. Nevertheless, the mechanism that facilitates initial control or contributes to the eventual reactivation of infection should provide critical information about what determines long-term protective immunity against an infection with M. tuberculosis.

**IS GENERATION OF ACQUIRED IMMUNITY DEFECTIVE IN THE SUSCEPTIBLE MOUSE STRAINS?**

A central question to be addressed is what causes the diminished CD4 T cell response in the lungs of M. tuberculosis-susceptible mice. Behar et al. hypothesized that slower dissemination of M. tuberculosis Erdman infection into the draining lymph nodes and spleen, observed in the highly susceptible C3H mouse strain, could delay the generation of an optimal immune response. This delay in T cell priming could permit early bacterial proliferation within the lungs, resulting in elevated bacterial burdens prior to the initiation of protective TH1-mediated immunity. This increased bacterial load may subsequently compromise long-term control. In support of this hypothesis, Musa et al. have shown that several of the mouse strains with increased susceptibility to infection (CBA/J, DBA/2, and A/J) showed delayed dissemination into the spleen in comparison with the resistant C57BL/6, BALB/c, and B10.A mouse strains following an aerosol infection with M. tuberculosis. In addition, cessation of bacterial growth within the lungs of CBA/J and DBA/2 mice occurred at a higher bacterial load than that observed in the C57BL/6 mouse strain, again supporting the notion that delayed T cell priming allows M. tuberculosis to grow unchecked within the lung for longer before eventually being controlled. Dissemination of M. tuberculosis into the draining lymph nodes and other secondary lymphoid tissues may, therefore, be of particular relevance for the initiation of an immune response within the lung. A significant delay between initial infection and recruitment of immune cells to the lung may lead to the escalating bacterial loads observed in the highly susceptible mouse strains and the accelerated mortality within this particular cluster of strains.

Vaccination studies using reactivation-susceptible or -resistant mouse strains provide evidence that delayed priming of the immune system is not the only deficiency that can account for the reduced generation of protective immunity within the lung. Following vaccination with M. bovis BCG, C57BL/6 mice rapidly generated protective immunity against subsequent challenge with M. tuberculosis H37Rv, resulting in greater than 1 Log fewer bacteria within the lung and spleen compared with non-vaccinated mice. Interestingly, vaccination of CBA/J and DBA/2 mouse strains with M. bovis BCG resulted in the generation of protective immunity against subsequent intravenous infection with M. tuberculosis within the spleen that was comparable to that measured in the reactivation-resistant C57BL/6 mouse strain. In contrast, vaccination with M. bovis BCG did not enhance the generation of protective immunity against intravenous infection with M. tuberculosis in the lung in the reactivation-susceptible strains tested. The critical element of these studies was the delivery of M. tuberculosis by the intravenous route. This route of administration results in an immediate systemic infection and circumvents the need to traffic cells between the lung and draining lymph nodes to prime the immune response. In this model, reactivation-susceptible mice were fully capable of priming the immune response and generating protective immunity in the spleen; however, the capacity to generate protective immunity within the lung remained impaired. As might be anticipated, vaccination with M. bovis BCG also failed to protect CBA/J mice.
against a subsequent aerosol challenge with *M. tuberculosis*. These findings parallel the results from *in vitro* studies of cultured primary splenocytes in which isolated spleen cells from reaction-susceptible mouse strains were fully capable of producing antigen-specific IFN-γ at comparable or often higher levels than cells from C57BL/6 mice following *M. tuberculosis* aerosol infection. In contrast, the capacity of isolated lung cells from reaction-susceptible mice to produce IFN-γ was substantially impaired. These studies demonstrate that a delayed dissemination of *M. tuberculosis* from the lung to the lymph nodes is not solely responsible for the inadequate generation of protective immunity within the lung. Susceptibility to infection with *M. tuberculosis* appears to be linked to the generation of specific pulmonary immunity and suggests that reaction-susceptible mice may be deficient in their capacity to recruit or maintain antigen-specific T cells within the lung.

### Do T Cells Fail to Follow Directions?

The vaccination data, in combination with histological examination of lung tissue, indicate that although reactivation-prone mouse strains could generate sufficient systemic protective immunity, these same mouse strains had inadequate migration or expansion of antigen-specific T cells into the infected lung. It is unclear whether this failure to migrate into the lung tissue is a deficiency within the T cell population itself or a failure to recruit T cells to the lung, perhaps by the inadequate secretion of chemokines. During infection with *M. tuberculosis* Erdman, a flow cytometric comparison of T cells isolated from the spleen or lung of reactivation-resistant and -susceptible mouse strains revealed that T cells from the reactivation-susceptible mouse strains consistently failed to increase the density of CD11a (LFA-1 α chain) and CD54 (ICAM-1) on the cell surface. Indeed, reduced expression of the adhesion molecules CD11a and CD54 could be observed on T cells isolated from both the spleen and the lung throughout the entire infection period studied (up to 120 days post-infection). The expression of CD11a and CD54 on the surface of T cells can facilitate the migration of cells through the blood vessel wall and into sites of inflamed tissue. In the absence of either adhesion molecule, T cells would be unable to efficiently traffic to the local site of infection. This failure could compromise the ability of reactivation-susceptible mice to control and express long-term containment of an infection with *M. tuberculosis* within the lung. Interestingly, despite the decreased expression of CD11a and CD54 on the surface of T cells, reactivation-susceptible mouse strains expressed protective immunity within the spleen, suggesting that additional deficiencies in the expression of adhesion molecules that recruit T cells specifically to the lung may yet be identified.

### Are T Cells Misdirected?

An alternative hypothesis, and one that implicates unique lung-specific events, is that reduced T cell infiltration into the lungs of susceptible mice is due to impaired cellular migration and altered infiltration into infected lung tissue. Cellular recruitment is mediated by chemokines which are secreted into the local tissue matrix and guide incoming immune cells. Insufficient or inappropriate chemokine secretion within the lung could alter the recruitment of protective immune cells into the infected tissues and contribute to the reduced number of lymphocytes within the lung of reactivation-susceptible mice. An absence or reduction in chemokine signal within the lungs of reactivation-susceptible mouse strains may well account for the differences in granuloma organization and structure as well as decreased numbers and location (perivascular and peribronchiolar) of T cells within the lung tissue during infection with *M. tuberculosis*.

Cardona et al. investigated the differential expression of chemokines within the lungs of C57BL/6 (reactivation resistant) and DBA/2 (reactivation susceptible) mice over the course of a 22-week infection. These studies reported a general trend for increased CCL7 (MCP-3) and CXCL2 (MIP-2) mRNA expression within the lungs of DBA/2 mice, and the authors suggested that these chemokines may contribute to the superior influx of monocytes and neutrophils into the lung tissue. More significant, perhaps, was the observation that lung tissue from DBA/2 mice had reduced levels of mRNA for CCL5 (RANTES), a chemokine that has been associated with the generation of a TH1 response. These contrasting chemokine profiles may account for the differential cellular recruitment of cells into the lungs of DBA/2 mice during infection with *M. tuberculosis*. Interestingly, reduced CCL5 mRNA expression has also been observed in the CBA/J mouse strain (Turner et al., unpublished observations), identifying a potential link between a deficiency in CCL5 and susceptibility to reactivate an infection with *M. tuberculosis*. Cardona et al. also reported a decreased production of ICAM-1 mRNA within the lungs of DBA/2 mice, further supporting the association between reduced ICAM-1 expression on T cells and the reactivation-susceptible mouse strains. In further support that cell trafficking is critical to the containment of an infection with *M. tuberculosis* is the observation that mice deficient in complement component 477.
C5 failed to form adequate granuloma, which was associated with reduced production of the chemokines MIP-2 (CXCL2), IP-10 (CXCL10), and MCP-1 (CCL2), and a failure to limit the growth of M. tuberculosis Erdman within the lung. These studies highlight the significance of complement components, and the downstream effects of their production, on the maintenance of M. tuberculosis infection. In contrast, however, mice deficient in complement receptor 3 did not exhibit any deficiencies in their ability to control an infection with M. tuberculosis. Investigation into the role of complement components on the susceptibility of mice to infection with M. tuberculosis warrants further investigation.

The relationship between chemokine secretion and the expression of adhesion molecules on the surface of T cells appears to dramatically influence the outcome of an infection with M. tuberculosis. Reactivation-susceptible mouse strains generated an altered chemokine profile within the lungs in response to infection with M. tuberculosis compared when reactivation-resistant mice, and T cells failed to increase the expression of adhesion molecules that can facilitate cellular migration. Reduced RANTES expression in particular may contribute to the failure of T cells to migrate into the granuloma; however, the substantial redundancy in the chemokine network may prove to be a challenge when interpreting these data further. Nevertheless, these studies draw attention to the importance of cellular recruitment into the lung and the formation of compact and highly organized granulomas.

**ALTERED MACROPHAGE RESPONSES IN REACTIVATION-SUSCEPTIBLE MICE**

The increased number of macrophages within the lungs of reactivation-susceptible mice and the altered chemokine profiles of DBA/2 mice highlight the role of macrophages in the outcome of infection; however, no studies to date have directly addressed macrophage function in vivo. An alternative strategy is to investigate macrophage-derived cytokine and chemokine production in vitro. Keller et al. investigated the response of bone marrow-derived macrophages from reactivation-resistant (C57BL/6 and BALB/c) and -susceptible (CBA/J and DBA/2) mouse strains during infection with M. tuberculosis H37Rv. Initial findings showed that macrophages from all four strains tested were equally capable of taking up M. tuberculosis, suggesting that phagocytic capabilities were not impaired in macrophages obtained from reactivation-susceptible mice. Subsequent analysis of cytokine production was, however, altered, indicating that macrophages derived from reactivation-resistant and -susceptible strains respond differently to infection. Macrophages from susceptible strains produced far less TNF and IL-10 than resistant strains, but had an increased production of LIX (a molecule homologous to the human chemokine receptor 5)46. The increase production of LIX in macrophages derived from reactivation-susceptible mouse strains was supported by increased message for this molecule, measured by microarray technology. Microarray analysis identified several additional genes that were also increased in macrophages isolated from reactivation-susceptible strains, which included genes for several chemokines and chemokine ligands: CCL4 (MIP-1β), CCL3 (MIP1α), CCL7 (MCP3), and CCR1 (the receptor for CCL3, CCL5, and CCL7)46. The majority of genes identified in vitro using bone marrow-derived macrophages have not yet been confirmed in vivo; however, the increased expression of CCL7 is at least supported by the studies of Cardona et al.12, as previously described above. In vitro macrophage studies again identified an altered chemokine profile in reactivation-susceptible mouse strains, indicating that the appropriate secretion of chemokines may be integral to the development of protective immunity. It is currently unknown why macrophages from various mouse strains respond so differently to infection with M. tuberculosis. An investigation into the expression of cell surface receptors that are involved in pathogen recognition or uptake may identify significant differences in host response between reactivation-resistant and -susceptible mouse strains. One specific example that has already been investigated is possession of the Nramp gene. The Nramp' phenotype (defined as an increased resistance to M. bovis BCG infection65) is, interestingly, expressed by reactivation-susceptible mouse strains66, 63. Studies of susceptibility to M. tuberculosis using Nramp congenic mice have revealed that possession of the Nramp' phenotype is not solely responsible for the reactivation of an infection57, 65. The Nramp' phenotype might, however, contribute to disease progression when paired with other susceptibility genes that can be found in reactivation-susceptible mouse strains or people.

**ARE IMMUNE RESPONSES TURNED OFF IN THE LUNG?**

The in vitro studies described above showed that macrophages from reactivation-susceptible mouse strains produced substantially less IL-10 in response to infection with M. tuberculosis than macrophages from reactivation-resistant strains66. These findings suggest that reactivation-susceptible mice may be unable to down-regulate an inflammatory response,
and this might account for the increased inflammation within the lungs of CBA/J and DBA/2 mice during the late stages of infection. The observed decreased IL-10 production in vitro does not, however, correlate with in vivo findings, as macrophages within the lungs of CBA/J mice contained abundant IL-10 during infection with M. tuberculosis Erdman. These conflicting data may simply reflect the different models being investigated (in vitro vs in vivo) or, alternatively, the data could identify an altered response from macrophages that have been chronically infected with M. tuberculosis. Increased production of IL-10 could be an important indicator of susceptibility to reactivation TB; however, it is important to determine whether IL-10 production is elevated in response to increasing tissue damage (in an attempt to dampen an inflammatory response brought about by increasing bacterial load) or that increased IL-10 production down-regulates inflammation and facilitates reactivation of infection. To determine whether the increased production of IL-10 observed in vivo could be directly related to the reactivation of infection, a model of IL-10 over-expression has been investigated. C57BL/6 mice (a naturally resistant strain) engineered to express the IL-10 gene under control of the IL-2 promotor (IL-10 transgenic mice) were infected with M. tuberculosis Erdman. M. tuberculosis infection stimulates the production of IL-2, which in this transgenic mouse model resulted in the associated secretion of IL-10. Following an infection with M. tuberculosis, IL-10 transgenic C57BL/6 mice showed evidence of reactivation of infection in a time-frame similar to CBA/J mice. These data suggest that increased production of IL-10 in the lungs is conducive to reactivation of infection. As discussed above, IL-10 is a potent anti-inflammatory cytokine and it is possible that IL-10 is produced in abundance in CBA/J mice to quench an overwhelming inflammatory response. CBA/J mice do develop substantial pulmonary lesions during infection with M. tuberculosis, and IL-10 may be a byproduct of an exuberant inflammatory response. The over-expression of IL-10 on a naturally resistant C57BL/6 mouse background, however, proved that IL-10 production could drive the reactivation phenotype within the lung in the absence of any confounding factors responsible for stimulating IL-10 production. Increased production of IL-10 can clearly drive reactivation TB, and this finding may be highly relevant to co-infection studies of M. tuberculosis and human immunodeficiency virus (HIV). The close relationship between these two pathogens suggests synergy between M. tuberculosis and HIV; it is particularly interesting to note that infection with HIV is associated with increased IL-10 production. Although the low CD4 T cell numbers and diminished TH1 immune response influence reactivation of TB in HIV-positive individuals, it can be hypothesized that HIV-driven IL-10 production can also directly influence the capacity of the host to contain an infection with M. tuberculosis.

The anti-inflammatory properties of IL-10 can account for several of the parameters associated with reactivation of an infection with M. tuberculosis. Reduced IL-12 and TNF production, altered chemokine expression, and decreased antigen-specific IFN-γ production can all be attributed to the IL-10-mediated suppression of the macrophage response in vivo. Indeed, C57BL/6 IL-10 transgenic mice had reduced numbers of CD4 T cells within the lungs, failed to produce antigen-specific IFN-γ as efficiently as wild-type C57BL/6 mice, and developed abnormal granulomas that resembled the microscopic lesions typically observed in susceptible CBA/J mice. In addition, T cells isolated from the lungs of IL-10 transgenic C57BL/6 mice expressed less CD11a and CD54 than wild-type C57BL/6 mice, suggesting that IL-10 production may directly influence T cell migration. The increased production of IL-10 in the naturally reactivation-resistant C57BL/6 mouse strain, therefore, recapitulated the susceptible CBA/J phenotype. These findings make a compelling argument for IL-10-mediated down-regulation of immune responses within the lungs of reactivation-susceptible mice and an association of this cytokine with reactivation of infection. IL-10 is, therefore, a correlate of susceptibility to reactivate an infection with M. tuberculosis.

An alternative perspective is that the increased susceptibility to reactivate an infection with M. tuberculosis is driven by TH2 cytokines, potentially mediated by glucocorticoid production. Rook et al. hypothesized that the production of cortisone during infection with M. tuberculosis can dampen an inflammatory response, reducing IFN-γ and IL-12 and increasing the production of TH2 cytokines and IL-10. Indeed, the ratio between dehydroepiandrosterone and cortisol, particularly in regard to skewing of the immune TH1/TH2 repertoire, appears to be a critical factor in the progression of TB disease. Studies again implicate IL-10 in the progression of disease and link the production of this cytokine to adrenocortical production of glucocorticoids and their precursors. While there is no immediate evidence for increasing TH2 immunity in the lungs of reactivation-susceptible mice, Actor et al. demonstrated that the susceptible A/J mouse strain produced higher levels of serum cortisol than the reactivation-resistant C57BL/6 mice when injected with M. tuberculosis cord factor, again supporting a role for glucocorticoids in susceptibility to TB.
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compared with controls, suggesting that a reduced capacity to produce antigen-specific IFN-γ in peripheral blood cultures from TB patients also have decreased IFN-γ production are reportedly increased in TB patients. It is interesting to note that a reduced anti-inflammatory response correlates with disease progression in man. The murine model of TB has been an invaluable tool for characterizing the immunologic response to M. tuberculosis infection. The study of different inbred mouse strains can also provide insight into the heterogeneous immune responses that are observed in the diverse human population. Inbred mouse strains succumb to infection with M. tuberculosis in variable, but predictable, periods of time. This variability offers an opportunity for researchers to investigate the different immune criteria that can influence disease progression. Several common themes emerge between different mouse strains. One is the development of large, coalescing, macrophage-dominated lung lesions with tissue destruction and necrosis. A second and highly reproducible trend is the reduced capacity of T cells from reactivation-prone strains to produce IFN-γ. Beyond these similarities lies a multitude of different immune parameters that appear to influence disease progression. The CBA/J mouse strain highlights the role of immunosuppressive cytokines in disease progression; studies using the A/J strain have identified a specific deficiency in complement component C5. Additionally, altered chemokine production and cellular migration appear to be a factor in adequate control of infection. Such variable results between mouse strains should not be thought of as a failure to reach consensus on immunological markers of susceptibility, but rather windows into the diversity of immunological responses and mechanisms that can influence disease pro-

PARALLELS TO HUMAN TB

The murine model of TB has been an invaluable tool for the identification of immune mechanisms that are critical for the containment of an infection with M. tuberculosis. These findings have led to the development of therapies to treat multi-drug-resistant TB and a better understanding of why drugs such as the TNF inhibitors perpetuate reactivation of an infection with this pathogen. These highly relevant discoveries in the mouse model have been integral in the delineation of immunity to infection with M. tuberculosis, despite the obvious limitations of the mouse model to mimic latent infection in man. In this regard, the murine model of TB can be viewed as a model of chronic infection in which the period of reactivation has parallels to disease progression in man.

One specific parallel to human studies is the reduced capacity of all reactivation-prone mouse strains to produce IFN-γ. IFN-γ is critical for the control of M. tuberculosis infection in mice, and individuals that have defects in IFN-γ signaling are more susceptible to mycobacterial diseases. In addition, peripheral blood cultures from TB patients also have decreased capacity to produce antigen-specific IFN-γ compared with controls, suggesting that a reduced IFN-γ response correlates with disease progression. It is interesting to note that a reduced antigen-specific IFN-γ response, or reduced capacity to produce IFN-γ, was reported in all reactivation-susceptible mouse strains tested. Whether the decrease in IFN-γ production seen in TB patients is due to sequestration of antigen-specific T cells to the lung is not clear from human studies; however, decreased reactivity of lung cells from reactivation-susceptible mice suggests that sequestration may not account for such a loss of reactivity in the blood and that a general decline in IFN-γ production may occur in both the periphery and the lung.

Further parallels between the murine model and man can be demonstrated with regard to the increased production of immunosuppressive cytokines. Both transforming growth factor-β (TGF-β) and IL-10 production are reportedly increased in TB patients, highlighting the association of immunosuppressive cytokines and disease progression that we have reported in mice. Whether TGF-β and IL-10 are produced in an attempt to dampen inflammation brought about by an escalating bacterial load within the lungs in man is currently unclear. However, murine studies suggest that a predisposition to produce immunosuppressive cytokine can facilitate reactivation of TB and it may be beneficial to explore the relationship between IL-10 and TGF-β in man in more detail.

In particular, Verbon et al. have shown that IL-10 levels are significantly elevated in the serum of patients with active TB compared with both treated TB patients and with healthy contacts. In addition, increasing levels of the M. tuberculosis antigen CFP32 in the sputum of TB patients (hypoththesized to indicate increased bacterial burden in the lung) correlated with sputum IL-10 production. The impact of IL-10 production, however, was best identified in a study carried out in Cambodia. Culture of peripheral blood cells from a subpopulation of TB patients with M. tuberculosis purified protein derivative (PPD) resulted in elevated IL-10 production and reduced IFN-γ production in vitro. This elevated IL-10 production correlated with a persistently negative (or anergic) skin test response. Skin test responsiveness to other infectious agents was retained, demonstrating an association between IL-10 production and a persistent anergic response to PPD. IL-10 production is, therefore, also associated with susceptibility to TB in man, and these findings emphasize the significance of exploring other mouse strains in our search to understand susceptibility to infection with M. tuberculosis.

CONCLUSIONS

The mouse model of human TB provides a powerful tool for characterizing the immunologic response to M. tuberculosis infection. The development of large, coalescing, macrophage-dominated lung lesions with tissue destruction and necrosis. A second and highly reproducible trend is the reduced capacity of T cells from reactivation-prone strains to produce IFN-γ. Beyond these similarities lies a multitude of different immune parameters that appear to influence disease progression. The CBA/J mouse strain highlights the role of immunosuppressive cytokines in disease progression; studies using the A/J strain have identified a specific deficiency in complement component C5. Additionally, altered chemokine production and cellular migration appear to be a factor in adequate control of infection. Such variable results between mouse strains should not be thought of as a failure to reach consensus on immunological markers of susceptibility, but rather windows into the diversity of immunological responses and mechanisms that can influence disease pro-
gression in the highly heterogeneous human population. Phenotypic patterns of immune dysfunction in specific inbred strains of mice may identify clear correlates that correspond to a subset of individuals that are infected with *M. tuberculosis* and identify immune responses that can ultimately lead to disease progression. Different mouse strains, particularly those that are more susceptible to infection with *M. tuberculosis*, are important for the discovery of immune responses that correlate with progression of TB and can aid in the identification and treatment of patients likely to develop significant pulmonary disease.

**References**


