Published in final edited form as:

Exp Gerontol. 2018 May ; 105: 32–39. doi:10.1016/j.exger.2017.12.021.

Tuberculosis in the elderly: Why inflammation matters

Tucker J Piergallini^{1,2} and Joanne Turner¹

¹Texas Biomedical Research Institute, San Antonio, TX 78227

²College of Medicine, The Ohio State University, Columbus, OH 43210

Abstract

Growing old is associated with an increase in the basal inflammatory state of an individual and susceptibility to many diseases, including infectious diseases. Evidence is growing to support the concept that inflammation and disease susceptibility in the elderly is linked. Our studies focus on the infectious disease tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (*M.tb*), a pathogen that infects approximately one fourth of the world's population. Aging is a major risk factor for developing TB, and inflammation has been strongly implicated. In this review we will discuss the relationship between inflammation in the lung and susceptibility to develop and succumb to TB in old age. Further understanding of the relationship between inflammation, age, and *M.tb* will lead to informed decisions about TB prevention and treatment strategies that are uniquely designed for the elderly.

Keywords

aging; inflammation; tuberculosis; lung; immune cells

1. Introduction

Increasing age is associated with a multitude of changes throughout the body including the accumulation of DNA damage, loss of tissue function, and reduced cognitive function (Bowen and Atwood 2004; Carvalho et al. 2014). In addition to increased risk of age-associated diseases such as cancer, cardiac disease, Alzheimer's, and/or an associated loss of mobility/independence (Lin and Woollacott 2005; Lopez-Otin et al. 2013; Niccoli and Partridge 2012), the elderly are also more susceptible to developing and succumbing to many infectious diseases (Gardner 1980; Meyer 2001). Changes in immune function with increasing age are considered to be a risk factor for susceptibility to infection in old age.

As individuals age, they experience changes in immune function that reflect maturation, dysfunction, and plasticity. The changes that occur in immunity with increasing age are

Corresponding author: Joanne Turner, Texas Biomedical Research Institute, 8715 W Military Dr, San Antonio, Texas 78227-5302.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: None

multifactorial and span both the innate and adaptive arms of the cellular and humoral immune system (Weiskopf et al. 2009) and these immune changes are associated with a poor response to and control of infectious agents (Hakim and Gress 2007). Much research has been previously focused on defining critical changes in innate and adaptive immunity with increasing age at the cellular and molecular level, whereas the influence of inflammation on innate and adaptive mechanisms at the systemic level has only been experimentally recognized more recently. As individuals age they experience an increase in basal inflammation (Franceschi et al. 2000), now recognized as an event called inflammaging. Inflammatory cytokines, including TNF and IL-6, are associated with increased risk for many diseases including sarcopenia, osteoarthritis, and many infectious diseases (Boe et al. 2017; Greene and Loeser 2015; Lloyd and Marsland 2017). However, the precise cause and effects of this process are still elusive (Baylis et al. 2013; Franceschi et al. 2000). What we do know is that inflammaging has far reaching effects throughout the body (Franceschi et al. 2000; Franceschi and Campisi 2014), but the mechanisms of its influence on immune responses and resulting increased susceptibility of aged persons to succumb to infectious diseases is currently still limited (Canan et al. 2014; Goldstein 2010).

The elderly are more susceptible to many infections, from those that are commonly diagnosed (influenza and pneumococcal pneumonia) (Bahadoran et al. 2016; Krone et al. 2014) to those considered more exotic (anthrax and SARS) (Leung et al. 2004; Lyons et al. 2004). Specific to the focus of this review, the elderly are more likely to develop and succumb to tuberculosis (TB) disease (Mehta and Dutt 1995; Zevallos and Justman 2003). The etiologic agent of TB, *Mycobacterium tuberculosis* (*M.tb*) is estimated to infect about one fourth of the world's population (Houben and Dodd 2016; WHO 2017). While the global burden of *M.tb* infection is significant, the majority of infected individuals are asymptomatic and harbor very low levels of bacteria in a disease state termed non-replicating persistence or latency (Zumla et al. 2013). It is only when indicators of poor health (HIV coinfection, malnutrition, diabetes, etc.) become apparent that approximately 5-15% of these latent individuals go on to develop active infection (reactivation TB) (Getahun et al. 2015). During reactivation, *M.tb* multiplies and the infected individual can become infectious, and may also succumb to symptoms associated with disease (Zumla et al. 2013). Increasing age is a major risk factor for developing and/or succumbing to TB, with over half of TB-related deaths occurring in those 50 years and older (Negin et al. 2015). Although reactivation of latent *M.tb* infection in the elderly contributes to many of the TB cases (Negin et al. 2015), it is also firmly established that the elderly are highly susceptible to developing TB if they become infected with *M.tb* when they are older (primary infection) (Rajagopalan 2001). Our laboratory seeks to determine the factors that contribute to the increased susceptibility of the elderly to develop or succumb to TB. Our primary research model is the aged mouse, due to its ease of use, availability, and relatively short lifespan. The focus of this review article will be on primary TB in old mice, although our laboratory has more recently initiated studies of age-associated reactivation TB in mice, as well as extended our studies to TB in elderly human subjects.

When adult mice are infected with *M.tb* by the aerosol route, they experience a period of unrestricted *M.tb* growth in the lung for approximately 14-21 days (Turner et al. 2002), after which the adaptive immune system is activated, *M.tb* growth is slightly reduced, and

infection is maintained at a stable chronic level for up to one year (Rhoades et al. 1997). This is associated with migration of innate and adaptive immune cells to the lung to control infection, in association with the formation of cellular aggregates called granulomas that prevent *M.tb* dissemination (Gideon et al. 2015; Orme and Basaraba 2014; Ramakrishnan 2012). This pattern of events is considered to reflect early *M.tb* infection in humans. Diagnostic tests for *M.tb* infection indicate a lag between predicted exposure and detection of immune responsiveness 4-6 weeks later (Lee et al. 2011; Winslow et al. 2008). Furthermore, many cell and cytokine responses that have been identified as critical for *M.tb* control in mice are also essential in humans (Feng et al. 2006; Flynn and Chan 2001; Means et al. 1999; Sanchez et al. 2010). In contrast to humans, however, mice do not reduce *M.tb* bacterial loads in the lung to low levels or develop a state of non-replicating persistence (Rhoades et al. 1997). This limitation has the most impact on long term studies where the high *M.tb* bacterial burden in mice substantially shortens their lifespan (Orme 1988; Rhoades et al. 1997). Similar to humans, TB disease in mice is associated with a loss of immune control and regrowth of *M.tb* to levels that cause tissue damage and impaired lung function. This disease state is accompanied by extensive local and systemic inflammation, characterized by significant weight loss and muscle wasting that has been linked to abundant TNF production (Harris and Keane 2010; Paton and Ng 2006; Quesniaux et al. 2010). Because TB is highly associated with a robust systemic inflammatory response, it is likely that age-associated inflammation further contributes to TB pathogenesis in elderly mice, and by extrapolation in elderly humans.

Studies of TB in mice, and specifically studies of aging and TB, have primarily used C57BL/6 or BALB/c mice, the background strains for most genetic knockout mice and strains that are available from commercial vendors at an older age. It has become increasingly recognized that studies of different inbred and outbred mice can more accurately reflect some of the diverse outcomes of *M.tb* infection in humans (Beamer and Turner 2005; Medina and North 1998; Smith et al. 2016). While alternates to C57BL/6 and BALB/c mice are still limited for aging studies, it is apparent that differences between C57BL/6 and BALB/c strains can account for some age-associated responses (Boehmer et al. 2005; Renshaw et al. 2002). In the context of *M.tb* infection, our group have used C57BL/6 and BALB/c mice interchangeably without impact on experimental outcomes (Turner et al. 2002).

We and others have previously established that old mice are quicker to succumb to primary *M.tb* infection compared to adult mice (Orme 1995; Vesosky and Turner 2005), which is associated with increasing *M.tb* bacterial burden in the lung at late stages of infection (Turner et al. 2002). However, despite this increased susceptibility to develop and succumb to TB sooner than adult mice, old mice can generate potent innate immune responses (Rottinghaus et al. 2009). Indeed, innate immunity is robust enough to restrict the early growth of *M.tb* in the lungs of old mice, which will be the focus of this review. While studies are ongoing in our laboratory to determine how inflammation can alter adaptive immune function in old mice, this aspect will be discussed only briefly in this review. Our focus here will be on how an established state of inflammation in old age can modify the first encounter that *M.tb* has with host cells and molecules within the pulmonary space, the initial site of infection.

2.1. The lung microenvironment

M.tb is primarily transmitted by aerosol droplets that are inhaled into the lung and either cleared via mechanical mechanisms or deposited into the bronchioles and alveolus where infection can be established. It is thought that the status of the lung environment at the time of infection with *M.tb* is an important factor in determining disease severity (Torrelles and Schlesinger 2017). Although the lung is the primary portal of entry for *M.tb*, the impact of the aging lung has only recently been considered as a factor that may define susceptibility to TB in the elderly.

The physical environment of the lung changes with age (Dyer 2012; Fragoso and Lee 2012) and makes the elderly more susceptible to many infections. The elderly experience a decreased lung elasticity and strength of respiratory muscles. Combined with lowered vital capacity (Dyer 2012), this can impair the expulsion of infectious agents through cough reflex, sneezing or breathing. Furthermore, increased incidence of fluid and/or solid aspiration into the lung with old age, and age-associated inflammatory disease such as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis (Akgun et al. 2012), make the elderly more likely to have a pulmonary environment that favors the establishment of infection, including *M.tb* infection.

Once *M.tb* has entered the bronchioles and alveolus, the bacterium resides within the lung mucosa that lines the alveolus, and in that mucosa the bacterium is exposed to soluble innate components (*i.e.* surfactant proteins, complement, hydrolases, antimicrobial peptides, antibodies, etc.) prior to encounters with resident structural (alveolar epithelium), resident innate (alveolar macrophage) and infiltrating innate (neutrophil, monocyte) cells that can determine the progress of *M.tb* in establishing infection. An inflammatory pulmonary environment in old age has the potential to modify each of these interactions between host cells and *M.tb*. Inflammaging has been historically defined as a change in cytokine levels in the circulation of an aged person, most notable being the increased levels of circulating pro-inflammatory cytokines such as TNF and IL-1 β (Franceschi and Campisi 2014). While we can make assumptions that inflammaging will also be evident in tissues, the presence and source(s) of inflammation in the aged lung has only recently been established by our group (Canan et al. 2014; Moliva et al. 2014).

2.2. Alveolar epithelial cells (AT) and alveolar lining fluid (ALF)

Lung mucosa or ALF is generated, secreted, and recycled by alveolar epithelial cells (ATs), and is essential for proper lung maintenance (Notter 2000). In the aged individual, senescent ATs lead to a decrease in lung recycling (Notter 2000) which in turn can drive a low level of inflammation in the lung (Reynolds 1987). With systemic inflammaging previously defined in the circulation (Franceschi et al. 2000), it is therefore reasonable to extrapolate that ALF in old age will also have an elevated inflammatory profile. Indeed, studies from our group (Moliva et al. 2014) have shown that ALF isolated from aged mice had significantly increased levels of TNF and IL-6 and a trend for increased IL-1 β . Importantly, this finding was similar in ALF from elderly human donors, confirming that inflammatory cytokines were also present in pulmonary fluids of humans in old age (Moliva et al. 2014).

The presence of increased inflammation within the lung mucosa was also highly associated with changes in multiple innate molecular defense mechanisms that could potentially influence the ability of *M.tb* to establish infection (Table 1). Surfactant proteins A and D (SP-A, SP-D) as well as components of the complement system, notably C3b, were found to be increased in aged mice and elderly human subject ALF (Moliva et al. 2014). In contrast, aged mice had decreased levels of lung hydrolytic enzymes (Moliva et al. 2014), which are capable of altering the *M.tb* cell wall and dictating its interaction with host cells. SP-A regulates apoptotic cell clearance, increases phagocytosis of *M.tb* by macrophages acting as an opsonin by direct interaction between SP-A and macrophage receptors, regulates inflammation and the oxidative response, and also regulates the expression of Toll-like receptors (TLRs) and the mannose receptor (MR) in human macrophages (Carlson et al. 2010; Torrelles et al. 2008). SP-D, on the other hand, reduces *M.tb* association with macrophages (Ferguson et al. 1999) and drives phagosome-lysosome fusion of those *M.tb* that are phagocytosed (Ferguson et al. 2006). With these important functions of SP-A and SP-D in mind, studies have shown that SP-A and SP-D can contribute to increased *M.tb* virulence by enhancing *M.tb* association to lung epithelial cells (Hall-Stoodley et al. 2006). Moreover, by activation of the classical and alternative complement system, C3 can opsonize *M.tb*, initiating phagocytosis by interaction with CR3 on macrophages (Ernst 1998; Ferguson et al. 2004). Lung hydrolases, on the other hand, can modify the cellular envelope of *M.tb*, changing interactions between *M.tb* and host cells (Arcos et al. 2011). These cell wall alterations have been shown to alter *M.tb* phagocytosis by macrophages and neutrophils, and allow these cells to significantly better control *M.tb* intracellular growth, minimizing inflammation and subsequently tissue damage (Arcos et al. 2015; Arcos et al. 2016; Scordo et al. 2017).

Altered levels of surfactant proteins, complement components and hydrolases may occur through dysregulation of their homeostatic production with increasing age or they may be altered to compensate for host-mediated changes in their function. Indeed, increased oxidation was observed in the ALF of aged mice (Moliva et al. 2014), suggesting that innate modulators of aged hosts may have undergone oxidative modifications that could limit their function. Moreover, surfactant lipid oxidation is also observed in surfactant of elderly individuals, where a decrease of dipalmitoylphosphatidylcholine (DPPC) and increased POPC (oxidative form of DPPC) was observed (Moliva et al. 2014). Surfactant lipids such as DPPC are critical for SP-A function. SP-A binds to DPPC but not to POPC suggesting that soluble SP-A may be susceptible to oxidation in ALF from the elderly.

Changes in innate immune molecule levels in the lung in an aging individual are just being elucidated and it is currently unknown whether changes in the amounts relative to young mice or humans translates to modified function. We can speculate that altered levels or function of complement, surfactant proteins, and hydrolases in the lung environment as we age will modify how *M.tb* associates with different receptors on resident epithelial cells and macrophages or infiltrating neutrophils, triggering different uptake mechanisms, and ultimately resulting in altered trafficking patterns upon entry inside the macrophage. This likely affects the long-term survivability of *M.tb* within the macrophage. This, coupled with intrinsic differences in aged macrophage function that we will describe below, may amplify the increased vulnerability of the aged to *M.tb*.

3. Alveolar and pulmonary macrophages

Increased inflammation and the altered molecular innate environment in the lung is likely to cause significant functional effects on the resident cells that are the first to encounter *M.tb*. The alveolar macrophage is the primary niche for *M.tb* survival during infection, and one of the first host cells to come into contact with *M.tb* (Orme et al. 2015). Initial recognition of *M.tb* is accomplished by phagocytic receptors (e.g. complement receptors, mannose receptor, and SP-A/D) and signaling receptors (e.g. Toll-like receptors) (Ferguson et al. 1999; Gaynor et al. 1995; Guirado et al. 2013; Henning et al. 2008; Kang and Schlesinger 1998), leading to a specific uptake pathway which can modify the ability of *M.tb* to survive and persist in host cells. Upon recognition of *M.tb*, the bacterium will typically be phagocytosed and internalized, directing the bacterium into a phagosome/early-endosomal compartment (Philips and Ernst 2012; Sasindran and Torrelles 2011). The macrophage will attempt to fuse the phagosome with the lysosome, in a process called phagosome-lysosome (P-L) fusion with the goal to kill *M.tb* (Schlesinger et al. 2008). However, *M.tb* has developed ways to prevent P-L fusion (Schlesinger et al. 2008), allowing it to exist in an early-endosome-like compartment long-term (McDonough et al. 1993). Recent evidence has shown degradation of the phagosome can occur, allowing *M.tb* to escape into the cytosol for further replication (Simeone et al. 2012; van der Wel et al. 2007). Overall, *M.tb* survives by blocking P-L fusion or escaping from phagosomes to the cytosol. Meanwhile, stimulation of autophagy is shown to suppress *M.tb* intracellular survival (Gutierrez et al. 2004). Other studies, however, indicate that autophagosomes could also be a niche for *M.tb* intracellular survival (Arcos et al. 2016). Concomitant with *M.tb* uptake, autophagy, and P-L fusion, resident macrophages respond to infection by secreting numerous inflammatory cytokines and chemokines (Flynn and Chan 2001; Mendez-Samperio 2008; Sasindran and Torrelles 2011) that serve to attract infiltrating innate cells and to activate cells of the adaptive immune system. It is anticipated that both receptor mediated uptake and recognition, and effector functions of macrophages will be changed in old age.

In general, it is believed that innate cellular mechanisms are dysfunctional and/or reduced in old age (Boe et al. 2017). However, the studies regarding inflammatory cytokine production are contradictory, with some showing myeloid cells having decreased production of inflammatory cytokines (Boehmer et al. 2004; Boehmer et al. 2005; Chelvarajan et al. 2005; Renshaw et al. 2002), while others show a more robust production of cytokines both in local regions (Turnbull et al. 2009) and by monocytes (Gomez et al. 2007) in response to immune insult. Decreases in cytokine secretion may be explained by altered TLR function with age. Renshaw et al. (2002) showed that decreased TLR expression on macrophages correlated to lowered inflammatory cytokine production after immune stimulation. However, Boehmer et al. (2005) showed decreased pro-inflammatory cytokine secretion by aged macrophages did not correlate with altered TLR expression, instead correlating with altered TLR-signaling components in the cell. Additionally, Boehmer et al. (2005) showed only certain pathways to be affected with age in the macrophage, with TLR2 and TLR4-related stimuli resulting in decreased cytokine secretion, whereas cytokine secretion by macrophages after IL-2 stimulation was not altered with age. Overall, the phagocytic, inflammatory, and migratory capacities of the macrophage are also shown to have significant changes with age, yet there

is no conclusive signature of the aging macrophage (Brandenberger and Muhlfeld 2017). These contradictory findings are likely due to different mouse strain genetics (Boehmer et al. 2005; Renshaw et al. 2002), the different sources of macrophages (bone marrow derived *versus* resident) (Linehan et al. 2014), types of immune stimuli (Boehmer et al. 2005; Shaw et al. 2011) and tissue location of macrophages (Stout et al. 2005). Because *M.tb* is primarily a pathogen of the lung, and especially with regard to initial infection, our studies have been focused on resident pulmonary macrophages.

Our group has shown that resident macrophages isolated from the lungs of naïve old mice are in an increased inflammatory state compared to young controls (Canan et al. 2014) (Table 1). Pulmonary macrophages from old mice had increased levels of IRGM-1, an autophagy controller (Feng et al. 2009), IRF-1, a regulator of cytokine production and apoptosis (Harada et al. 1989), and CIITA, the MHC-II transcriptional factor (Canan et al. 2014; Steimle et al. 1994). The increase in these inflammatory responsive genes occurred concurrently with increases in IFN- γ , TNF and IL-12 in whole lung homogenates (Canan et al. 2014) and from isolated lung macrophages from naïve mice (Rottinghaus et al. 2009; Vesosky et al. 2006b; Vesosky et al. 2009). Changes in IL-12 or TNF production in the lung during *M.tb* infection in old age are highly relevant to *M.tb* pathogenesis. TNF is essential for macrophage activation and recruitment to the infection site (Lin et al. 2007) and mice receiving TNF blocking antibodies succumb to the infection quickly and cannot form granulomas (Flynn et al. 1995a). IL-12 is produced by macrophages in the lung as a response to *M.tb* infection (Ladel et al. 1997b), and is essential to Th1 mediated immunity and long-term control (Cooper et al. 1997; Flynn et al. 1995b). We have also demonstrated that several other inflammatory cytokines (IL-6, and IL-1 β) are elevated in macrophages from the naïve aged lung (Canan et al. 2014). The use of gene-disrupted mice has shown that IL-6 (Ladel et al. 1997a) and IL-1 β (Juffermans et al. 2000; Yamada et al. 2000) are necessary for *M.tb* control, with IL-6 deficient mice showing less pro-inflammatory cytokine secretion and a significantly shortened survival (Ladel et al. 1997a). While these studies may indicate that old mice have beneficial potent inflammatory responses after infection with *M.tb*, the long term consequences of inflammation are likely to impact granuloma formation and maintenance of chronic infection (Aggarwal 2003; Johnson et al. 1998; Robinson et al. 2015). In sum, these data indicate that resident cells not only respond and become activated by the local inflammatory environment, but can themselves directly contribute to the local inflammatory environment at basal levels.

We have also determined how macrophages from the lungs of old mice respond upon infection with *M.tb* both *in vitro* and *in vivo*. *In vitro* *M.tb* infection of CD11c+ cells (a marker of alveolar macrophages) purified from the lungs of naïve old mice led to similar IL-12 and TNF production as cells from young mice (Rottinghaus et al. 2010). Perhaps more significant however, CD11c+ cells isolated from old mice that had been infected with *M.tb* *in vivo* also secreted comparable, or moderately elevated, IL-12 and TNF levels compared to CD11c+ cells from young mice (Rottinghaus et al. 2010). Similar magnitude of cytokine production between macrophages from old and young mice to *M.tb* infection suggests that responses may be comparable. However, our group has demonstrated that TLR expression and signaling responses are altered in old age (Rottinghaus et al. 2010). Specifically, CD11c+ cells from the lungs of old mice could secrete IL-12 and TNF in response to *M.tb* infection

in a TLR2 independent manner, in contrast to our finding that CD11c+ cells from the lungs of young mice relied on TLR2 signaling to generate up to 75% of all cytokine production. This is significant because TLR2 is the dominant TLR that recognizes lipoproteins/lipopeptides on *M.tb* and stimulates cytokine release (Kleinnijenhuis et al. 2011; Underhill et al. 1999). Cytokine secretion by CD11c+ cells from old mice was partially dependent on TLR4 and TLR9, indicating that compensation in receptor signaling can occur in old age. As it has been shown in young mice that the sequence of infection based on the first receptor an *M.tb* bacterium interacts with on a macrophage can cause better or worse outcome (Philips and Ernst 2012), we can speculate that different TLR usage in macrophages from old mice could lead to differential downstream events within the *M.tb* infected macrophage (*i.e.* altered signaling cascades and cell fate), and establish an infection state that promotes long term susceptibility.

The basal inflammatory environment in the lungs of old mice, and potentially altered receptor mediated recognition and responsiveness that we have described, also has a functional consequence on macrophage responses. Alveolar macrophages isolated from old mice and infected *ex vivo* with *M.tb* showed differences in attachment, internalization, and killing compared to young mouse controls (Canan et al. 2014). Macrophages isolated from the lungs of old mice had increased numbers of total *M.tb* infected cells while also showing increased amounts of P-L fusion, suggesting a greater ability for killing (Canan et al. 2014). In support of the concept that macrophages within the aged lung are in a pre-activated state, we found that pretreatment of macrophages with IFN- γ showed no changes in *ex vivo* P-L fusion events in macrophages isolated from old mice. This was in contrast to macrophages isolated from young mice that had an expected increase in P-L fusion events in response to IFN- γ (Canan et al. 2014). We linked the elevated *M.tb* binding/uptake and baseline (non-IFN- γ activated) P-L fusion seen in macrophages from old mice to an increased inflammatory environment by reducing inflammation in old mice using dietary supplementation of an anti-inflammatory agent. Lung macrophages isolated from old mice receiving an ibuprofen supplemented diet had reduced levels of total inflammatory cytokines in the lung relative to old naïve mice and reduced cytokine production from pulmonary macrophages in response to *ex vivo* *M.tb* challenge. Additionally, any differences in P-L fusion and killing relative to young mice were negated (Canan et al. 2014), supporting our finding that elevated inflammation drove the altered responses seen in old mouse macrophages infected *ex vivo* with *M.tb*.

It is apparent that inflammation drives an increased activation state in pulmonary macrophages in old mice and that macrophages in the lung further contribute to the inflammatory environment at basal state and in response to *M.tb* infection. This robust inflammatory response that is associated with increased P-L fusion in macrophages *in vitro* and production of IL-12 and IFN- γ *in vivo* is highly associated with, and likely contributes to, a reduced *M.tb* bacterial burden early in the lungs of old mice (Vesosky et al. 2006a; Vesosky et al. 2006b) that we have previously defined. This phenotype, that we have termed early resistance, is linked to the presence of resident CD8 T cells in the lungs of old mice.

4. Resident CD8⁺ T cells

Conventional T cells respond to *M.tb* via antigen-specific (MHC-TCR) recognition of *M.tb* peptides and subsequent activation and secretion of IFN- γ (Woodworth and Behar 2006). IFN- γ , secreted by antigen specific CD4 and CD8 T cells, has long been known as an essential cytokine for long-term control of *M.tb*, with memory CD4 T cells secreting more IFN- γ secretion than memory CD8 T cells (Henao-Tamayo et al. 2014; Kirman et al. 2016). However, in studies of long-term *M.tb* infection in mice, memory CD8 T cells can proliferate (Kamath et al. 2006) and secrete IFN- γ (Serbina and Flynn 2001) after rechallenge with *M.tb*, showing their propensity for a functional memory response. IFN- γ is essential for macrophage activation, enhancing phagocytosis and killing, and is strongly associated with stabilization of *M.tb* infection *in vivo* (Flynn et al. 1993). In old age, antigen specific CD4 and CD8 T cell responses are thought to decline in number/frequency or be delayed in their generation (Weiskopf et al. 2009), leaving a significant gap between early innate control and the recruitment of antigen specific T cells to the tissue site to control infection. Furthermore, the memory CD8 T cell population shows distinct changes with age. There is a reduction in the proportions of naïve CD8 T cells relative to memory CD8 T cells in aged hosts (Goldrath et al. 2000; Saule et al. 2006). Despite the increased total percentage of memory CD8 T cell numbers, CD8 T cells in old age have reduced diversity of both the naïve and memory CD8 T cell receptor repertoire (Callahan et al. 1993; LeMaout et al. 2000; Posnett et al. 1994), which has been linked to poor immune responses of aged hosts to vaccines and viral infections (Blackman and Woodland 2011; Messaoudi et al. 2004; Yager et al. 2008). Chronic infection with cytomegalovirus (CMV) in humans and murine CMV (mCMV) in mice may act as a driver of this decreased repertoire diversity as large numbers of CD8 T cells in latent CMV-infected elderly humans (Khan et al. 2002) and mCMV-infected aged mice (Cicin-Sain et al. 2012; Smithey et al. 2012) have been found to be viral epitope specific, possibly contributing to poor immune function of T cells in the aged. While known altered function of aged memory CD8 T cells should translate to reduced *M.tb* control, our group has shown that when old mice are infected with *M.tb* they express an early control of infection, or transient early resistance. This is mediated in part through enhanced innate immune functions as described above, but also through a novel mechanism that appears to repurpose memory CD8 T cells that are resident within the lung.

Early work from our group has shown that the early resistance of old mice to *M.tb* is associated with increased numbers of CD8 T cells and increased IFN- γ secretion in the lung (Turner et al. 2002; Turner and Orme 2004) with up to 70% of these IFN- γ secreting CD8 T cells being CD44^{hi}CD45RB^{lo}, typically characterizing memory T cells (Mahnke et al. 2013). Based upon prior studies that CD8 T cells can respond directly to a combination of IL-12, IL-18 and IL-2 cytokines during *Listeria monocytogenes* infection in young mice (Berg et al. 2003), we confirmed that lung suspensions or purified CD8 T cells from the lungs of naïve old mice could also secrete IFN- γ in response to IL-12, IL-18, and IL-2 (Vesosky et al. 2006b). Receptors to IL-12, IL-18, and IL-2 on CD8 T cells were also increased with age (Vesosky et al. 2006a; Vesosky et al. 2006b). Most significant however, was that CD8 T cells from old mice could secrete IFN- γ in response to IL-12 alone. IL-18 enhanced the secretion but only when in the presence of IL-12, and in contrast to studies by

Berg *et al*, IL-2 was unnecessary. We confirmed that IFN- γ secretion was specific to IL-12p70 and not IL-12p40 homodimers, monomers, or IL-23 (Vesosky et al. 2009). Therefore, we identified a resident population of CD44^{hi}CD45^{lo} CD8 T cells in the lungs of old mice that could secrete IFN- γ in response to the single cytokine IL-12. Combined with our findings that IL-12 is present and secreted by macrophages in the lung both basally and during initial *M.tb* infection (Canan et al. 2014), and that CD8 T cells purified from *M.tb* infected old mice have abundant mRNA for IFN- γ (Vesosky et al. 2009), it seems likely that CD8 T cells are responding to basal inflammation in old age and subsequently contributing to the enhanced control of early *M.tb* infection in old mice.

Extending on our studies of CD8 T cell function (Table 1), we examined the direct IL-12 signaling pathway in CD8 T cells from old mice, specifically looking at expression of the IL-12 receptor (IL-12R β 2) and phosphorylation of the IL-12 transcription factor STAT4 (pSTAT4) after IL-12 stimulation (Bacon et al. 1995). Expression of IL-12R β 2 was increased on CD8 T cells from old mice and CD8 T cells had increased pSTAT4 compared to CD8 T cells from young mice, even when cells were normalized for CD44^{hi} expression (Rottinghaus et al. 2009). Furthermore, pSTAT4 could be inhibited in the presence of a Jak inhibitor. Additional studies demonstrated that IFN- γ secretion in aged CD8 T cells was due to higher amounts of SET, an inhibitor of the phosphatase PPA2 (Trotta et al. 2007). With increased amounts of SET, aged CD8 T cells could phosphorylate more STAT4, leading to increased IL-12 signaling and IFN- γ secretion compared to CD8 T cells from young mice. This was further confirmed when CD8 T cells were treated with forskolin to overcome SET action where total IFN- γ levels, along with pSTAT4, were reduced (Vesosky et al. 2009). These data provide evidence for altered IL-12 receptor and signaling profiles in old age that contribute to IL-12 specific stimulation of CD8 T cells from old mice. They also identified a novel innate-like CD8 T cell population that could respond to a single cytokine to release IFN- γ , which most likely required the backdrop of elevated basal inflammation that is seen in old age.

In young mice, NK cells can secrete IFN- γ during early *M.tb* infection via stimulation with Th1 cytokines which prompted us to determine if CD8 T cells from old mice expressed NK cell receptors, and whether NK cells could also produce IFN- γ during early infection. More CD8 T cells from old mice expressed NK cell receptors than young (Vesosky et al. 2006a) but purification of CD8⁺ or DX5⁺ (NK) cells from *M.tb* infected mice demonstrated that CD8⁺ cells were the dominant source of IFN- γ mRNA in old mice. In contrast, NK cells from young mice had more IFN- γ mRNA than NK cells from old mice (Vesosky et al. 2009). We confirmed that CD8 T cells from old mice were functioning in the absence of MHC-I/cell contact by transwell studies or culture of CD8 T cells from old mice with *M.tb* infected macrophages from β 2m-KO mice (MHC-I deficient) which maintained an elevated IFN- γ secretion (Vesosky et al. 2009). Co-culture studies of CD8 T cells with macrophages from young or old mice (*M.tb* infected, IL-12 producing) also clearly showed that the CD8 T cell from old mice was the driver of IFN- γ production as *M.tb* infected macrophages from young or old mice could stimulate IFN- γ production from CD8 CD44^{hi} T cells from old mice, but not CD8 CD44^{hi} T cells from young mice. Only neutralization of IL-12 led to a significant reduction in IFN- γ secretion by CD8 T cells from old mice, confirming that CD8 T cells from old mice have innate-like properties and can respond directly to a single

cytokine, leading to IFN- γ release. That IL-12 is found in abundance in the lungs of old mice both basally and in response to *M.tb* infection makes this response highly relevant for the early control of *M.tb* infection in old age. Interestingly, there have been several other studies showing an early resistance of aged mice to bacterial and parasitic infections (Cooper et al. 1995; Ehrchen et al. 2004; Lovik and North 1985) and we suggest that these may also be mediated by an IL-12 driven CD8 T cell response.

5. Conclusions

The aged lung is in a basal inflammatory state, driven by unknown factors but likely related to environmental stimulation as well as endogenous changes in cell function with increasing age. Basal increases in inflammatory cytokines such as TNF, IL-6 and IL-1 β can be seen in lung fluids and also secreted by resident lung cells (Canan et al. 2014; Franceschi et al. 2000). Furthermore, innate immune molecules such as complement, hydrolases and surfactant proteins A and D among others are modified in their amounts (Moliva et al. 2014) and we can further speculate that they will be modified in their function. Numerous studies have shown the biological relevance of SP-A/D, complement, and/or hydrolases on the outcome of *M.tb* infection *in vitro* (Arcos et al. 2015; Arcos et al. 2011; Arcos et al. 2016) and *in vivo* (Moliva et al. 2017), and as *M.tb* enters the lung and encounters ALF in an elderly person there is an initial opportunity to be modified in a way that could impact uptake by macrophages and alter long term infection outcome.

Independent of ALF changes, resident macrophages in the lungs in old age are in a heightened activation state due to contact stimulus from inflammatory cytokines (Canan et al. 2014). This stimulation leads to macrophages further contributing to the inflammatory milieu through cytokine secretion, and also modifies their function with increased binding and uptake of *M.tb* and increased P-L fusion (Canan et al. 2014), along with compensatory TLR signaling pathways that may alter downstream functional events (Rottinghaus et al. 2010). Altered receptor expression likely leads to differential uptake and intracellular killing pathways that provide *M.tb* with a niche to establish long term infection. Changes in macrophage function likely also impacts how adaptive immunity is generated, an area that has not yet been fully investigated.

Finally, the inflammatory lung environment in old age also includes basal increases in Th1 cytokines (IL-12, IFN- γ) that we have shown to play a dominant role in early resistance to *M.tb* infection in old mice (Rottinghaus et al. 2009; Vesosky et al. 2006a; Vesosky et al. 2006b; Vesosky et al. 2009). Elevated age-related basal IL-12 secretion in the lung can stimulate resident CD8 T cells to secrete IFN- γ which in turn likely contributes further to the increased activation state of resident macrophages. The addition of *M.tb*, a potent IL-12 stimulator, to the lung further potentiates this relationship between IL-12 (from macrophages) and IFN- γ (from CD8 T cells) and results in a transient yet significant reduction in *M.tb* within the lung over the first 3 weeks of infection in old mice (Turner et al. 2002). This occurs at a time when adaptive immunity is thought to be delayed and may serve as a compensatory mechanism to buffer that delay. However, this early resistance phenotype is transient and old mice (and humans) are still at increased risk to develop and succumb to TB relative to younger subjects (Negin et al. 2015; Turner et al. 2002). Therefore, we must

also consider that inflammation and early resistance to *M.tb* infection is related to worsening long term outcome. Some mechanisms that could contribute have been discussed above, such as modified receptor-mediated uptake and trafficking of *M.tb* to alternate and protective intracellular compartments. We also propose that early resistance may indirectly lead to poor generation of long term adaptive immunity through the stimulation of anti-inflammatory networks. We and others (Cyktor et al. 2013a; Cyktor et al. 2013b; Moreira-Teixeira et al. 2017; Redford et al. 2010) have demonstrated that the presence of interleukin 10 (IL-10) during priming of the adaptive immune system can have negative outcomes on the long term containment of *M.tb* infection. This is an area that warrants further investigation. Finally, the susceptibility of the elderly to develop and succumb to TB may simply be a direct impact of increased inflammation at all stages of infection. *M.tb* is a potent stimulator of multiple inflammatory cytokines (Flynn and Chan 2001; Sasindran and Torrelles 2011) and overlaid onto a basal increase of inflammation associated with old age (Franceschi et al. 2000), may simply lead to overwhelming inflammation that results in tissue and muscle wasting, pulmonary cellular infiltration and systemic symptoms that feed into the environment that *M.tb* thrives in. In this scenario, the study and utilization of anti-inflammatory drugs is highly relevant, as our studies with ibuprofen supplementation in old mice have demonstrated (Canan et al. 2014).

Acknowledgments

This work was supported by National Institute of Health, National Institute on Aging program project grant P01 AG051428 to JT. We thank Jordi B. Torrelles, Shalini Gautam, and Varun Dwivedi for their review of the manuscript.

Abbreviations

<i>M.tb</i>	<i>Mycobacterium tuberculosis</i>
AT	alveolar epithelial cell
ALF	alveolar lining fluid
TB	tuberculosis
P-L	phagosome - lysosome
SP-A	surfactant protein - A
SP-D	surfactant protein - D
IL	interleukin
MHC	major histocompatibility complex
TLR	toll-like receptor

References

Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature reviews Immunology*. 2003; 3:745–756.

- Akgun KM, Crothers K, Pisani M. Epidemiology and management of common pulmonary diseases in older persons. The journals of gerontology Series A, Biological sciences and medical sciences. 2012; 67:276–291.
- Arcos J, Diangelo L, Scordo J, Sasindran J, Moliva J, Turner J, Torrelles J. Lung mucosa lining fluid modifies *Mycobacterium tuberculosis* to reprogram human neutrophil killing mechanisms. Journal of Infectious Diseases. 2015; 212:948–958. [PubMed: 25748325]
- Arcos J, Sasindran SJ, Fujiwara N, Turner J, Schlesinger LS, Torrelles JB. Human lung hydrolases delineate *Mycobacterium tuberculosis*-macrophage interactions and the capacity to control infection. J Immunol. 2011; 187:372–381. [PubMed: 21602490]
- Arcos J, Sasindran SJ, Moliva JI, Scordo JM, Sidiki S, Guo H, Venigalla P, Kelley HV, Lin G, Diangelo L, Silwani SN, Zhang J, Turner J, Torrelles JB. *Mycobacterium tuberculosis* cell wall released fragments by the action of the human lung mucosa modulate macrophages to control infection in an IL-10-dependent manner. Mucosal Immunol. 2016
- Bacon CM, Petricoin EF III, Ortaldo JR, Rees RC, Lerner AC, Johnston JA, O'Shea JJ. Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes. Proc Natl Acad Sci USA. 1995; 92:7307–7311. [PubMed: 7638186]
- Bahadoran A, Lee SH, Wang SM, Manikam R, Rajarajeswaran J, Raju CS, Sekaran SD. Immune Responses to Influenza Virus and Its Correlation to Age and Inherited Factors. Front Microbiol. 2016; 7:1841. [PubMed: 27920759]
- Baylis D, Bartlett D, Patel H, Roberts H. Understanding how we age: Insights into inflammaging. Longevity & Healthspan. 2013; 2:1–8. [PubMed: 24764515]
- Beamer GL, Turner J. Murine models of susceptibility to tuberculosis. Arch Immunol Ther Exp (Warsz). 2005; 53:469–483. [PubMed: 16407780]
- Berg RE, Crossley E, Murray S, Forman J. Memory CD8+ T cells provide innate immune protection against *Listeria monocytogenes* in the absence of cognate antigen. The Journal of experimental medicine. 2003; 198:1583–1593. [PubMed: 14623912]
- Blackman MA, Woodland DL. The narrowing of the CD8 T cell repertoire in old age. Curr Opin Immunol. 2011; 23:537–542. [PubMed: 21652194]
- Boe DM, Boule LA, Kovacs EJ. Innate immune responses in the ageing lung. Clinical and experimental immunology. 2017; 187:16–25. [PubMed: 27711979]
- Boehmer ED, Goral J, Faunce DE, Kovacs EJ. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. J Leukoc Biol. 2004; 75:342–349. [PubMed: 14634059]
- Boehmer ED, Meehan MJ, Cutro BT, Kovacs EJ. Aging negatively skews macrophage TLR2- and TLR4-mediated pro-inflammatory responses without affecting the IL-2-stimulated pathway. Mechanisms of ageing and development. 2005; 126:1305–1313. [PubMed: 16154177]
- Bowen RL, Atwood CS. Living and dying for sex. A theory of aging based on the modulation of cell cycle signaling by reproductive hormones. Gerontology. 2004; 50:265–290. [PubMed: 15331856]
- Brandenberger C, Muhlfeld C. Mechanisms of lung aging. Cell Tissue Res. 2017; 367:469–480. [PubMed: 27743206]
- Callahan JE, Kappler JW, Marrack P. Unexpected expansions of CD8-bearing cells in old mice. J Immunol. 1993; 151:6657–6669. [PubMed: 8258683]
- Canan CH, Gokhale NS, Carruthers B, Lafuse WP, Schlesinger LS, Torrelles JB, Turner J. Characterization of lung inflammation and its impact on macrophage function in aging. J Leukoc Biol. 2014; 96:473–480. [PubMed: 24935957]
- Carlson, TK., Brooks, M., Meyer, D., Henning, L., Murugesan, V., Schlesinger, L. Pulmonary innate immunity: Soluble and cellular host defenses of the lung. In: Marsh, C. Tridandapani, S., Piper, M., editors. Regulation of Innate Immune function. Kerala: Transworld Research Network; p. 2010
- Carvalho A, Rea IM, Parimon T, Cusack BJ. Physical activity and cognitive function in individuals over 60 years of age: a systematic review. Clinical interventions in aging. 2014; 9:661–682. [PubMed: 24748784]
- Chelvarajan RL, Collins SM, Van Willigen JM, Bondada S. The unresponsiveness of aged mice to polysaccharide antigens is a result of a defect in macrophage function. J Leukoc Biol. 2005; 77:503–512. [PubMed: 15629885]

- Cicin-Sain L, Brien JD, Uhrlaub JL, Drabig A, Marandu TF, Nikolich-Zugich J. Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog.* 2012; 8:e1002849. [PubMed: 22916012]
- Cooper AM, Callahan JE, Griffin JP, Roberts AD, Orme IM. Old mice are able to control low-dose aerogenic infections with *Mycobacterium tuberculosis*. *Infect Immun.* 1995; 63:3259–3265. [PubMed: 7642254]
- Cooper AM, Magram J, Ferrante J, Orme IM. 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. [PubMed: 9206995]
- Cyktor JC, Carruthers B, Beamer GL, Turner J. Clonal Expansions of CD8(+) T Cells with IL-10 Secreting Capacity Occur during Chronic *Mycobacterium tuberculosis* Infection. *PLoS ONE.* 2013a; 8:e58612. [PubMed: 23472214]
- Cyktor JC, Carruthers B, Kominsky RA, Beamer GL, Stromberg P, Turner J. IL-10 Inhibits Mature Fibrotic Granuloma Formation during *Mycobacterium tuberculosis* Infection. *J Immunol.* 2013b; 190:2778–2790. [PubMed: 23396944]
- Dyer C. The interaction of ageing and lung disease. *Chron Respir Dis.* 2012; 9:63–67. [PubMed: 22308556]
- Ehrchen J, Sindrilaru A, Grabbe S, Schonlau F, Schlesiger C, Sorg C, Scharffetter-Kochanek K, Sunderkotter C. Senescent BALB/c mice are able to develop resistance to *Leishmania* major infection. *Infection and immunity.* 2004; 72:5106–5114. [PubMed: 15322004]
- Ernst JD. Macrophage receptors for *Mycobacterium tuberculosis*. *Infect Immun.* 1998; 66:1277–1281. [PubMed: 9529042]
- Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, Wynn TA, Sher A. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with *Mycobacterium tuberculosis*. *J Immunol.* 2006; 177:7086–7093. [PubMed: 17082625]
- Feng CG, Zheng L, Lenardo MJ, Sher A. Interferon-inducible immunity-related GTPase Irgm1 regulates IFN gamma-dependent host defense, lymphocyte survival and autophagy. *Autophagy.* 2009; 5:232–234. [PubMed: 19066452]
- Ferguson JS, Martin JL, Azad AK, McCarthy TR, Kang PB, Voelker DR, Crouch EC, Schlesinger LS. Surfactant protein D increases fusion of *Mycobacterium tuberculosis*-containing phagosomes with lysosomes in human macrophages. *Infect Immun.* 2006; 74:7005–7009. [PubMed: 17030585]
- Ferguson JS, Voelker DR, McCormack FX, Schlesinger LS. Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J Immunol.* 1999; 163:312–321. [PubMed: 10384130]
- Ferguson JS, Weis JJ, Martin JL, Schlesinger LS. Complement protein C3 binding to *Mycobacterium tuberculosis* is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect Immun.* 2004; 72:2564–2573. [PubMed: 15102764]
- Flynn JL, Chan J. Immunology of tuberculosis. *Annu Rev Immunol.* 2001; 19:93–129. [PubMed: 11244032]
- Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med.* 1993; 178:2249–2254. [PubMed: 7504064]
- Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, Schreiber R, Mak TW, Bloom BR. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity.* 1995a; 2:561–572. [PubMed: 7540941]
- Flynn JL, Goldstein MM, Triebold KJ, Sypek J, Wolf S, Bloom BR. IL-12 increases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. *J Immunol.* 1995b; 155:2515–2524. [PubMed: 7650381]
- Fragoso CAV, Lee PJ. The aging lung. *Journal of Gerontology.* 2012; 67A:233–235.
- Franceschi C, Bonafe M, Valensin S, Olivieri F, De LM, Ottaviani E, De BG. Inflammaging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000; 908:244–254. [PubMed: 10911963]

- Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2014; 69(Suppl 1):S4–9.
- Gardner ID. The effect of aging on susceptibility to infection. *Reviews of infectious diseases*. 1980; 2:801–810. [PubMed: 6763306]
- Gaynor CD, McCormack FX, Voelker DR, McGowan SE, Schlesinger LS. Pulmonary surfactant protein A mediates enhanced phagocytosis of *Mycobacterium tuberculosis* by a direct interaction with human macrophages. *J Immunol*. 1995; 155:5343–5351. [PubMed: 7594549]
- Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, Den Boon S, Borroto Gutierrez SM, Bruchfeld J, Burhan E, Cavalcante S, Cedillos R, Chaisson R, Chee CB, Chesire L, Corbett E, Dara M, Denholm J, de Vries G, Falzon D, Ford N, Gale-Rowe M, Gilpin C, Girardi E, Go UY, Govindasamy D, A DG, Grzemska M, Harris R, Horsburgh CR Jr, Ismayilov A, Jaramillo E, Kik S, Kranzer K, Lienhardt C, LoBue P, Lonnroth K, Marks G, Menzies D, Migliori GB, Mosca D, Mukadi YD, Mwinga A, Nelson L, Nishikiori N, Oordt-Speets A, Rangaka MX, Reis A, Rotz L, Sandgren A, Sane Schepisi M, Schunemann HJ, Sharma SK, Sotgiu G, Stagg HR, Sterling TR, Tayeb T, Uplekar M, van der Werf MJ, Vandevelde W, van Kessel F, van't Hoog A, Varma JK, Vezhnina N, Voniatis C, Vonk Noordegraaf-Schouten M, Weil D, Weyer K, Wilkinson RJ, Yoshiyama T, Zellweger JP, Raviglione M. Management of latent *Mycobacterium tuberculosis* infection: WHO guidelines for low tuberculosis burden countries. *The European respiratory journal*. 2015; 46:1563–1576. [PubMed: 26405286]
- Gideon HP, Phuah J, Myers AJ, Bryson BD, Rodgers MA, Coleman MT, Maiello P, Rutledge T, Marino S, Fortune SM, Kirschner DE, Lin PL, Flynn JL. 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. [PubMed: 25611466]
- Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *The Journal of experimental medicine*. 2000; 192:557–564. [PubMed: 10952725]
- Goldstein DR. Aging, imbalanced inflammation and viral infection. *Virulence*. 2010; 1:295–298. [PubMed: 21178458]
- Gomez CR, Hirano S, Cutro BT, Birjandi S, Baila H, Nomellini V, Kovacs EJ. Advanced age exacerbates the pulmonary inflammatory response after lipopolysaccharide exposure. *Crit Care Med*. 2007; 35:246–251. [PubMed: 17133178]
- Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. *Osteoarthritis and cartilage*. 2015; 23:1966–1971. [PubMed: 26521742]
- Guirado E, Schlesinger LS, Kaplan G. Macrophages in tuberculosis: Friend or foe. *Semin Immunopathol*. 2013; 35:563–583. [PubMed: 23864058]
- Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell*. 2004; 119:753–766. [PubMed: 15607973]
- Hakim FT, Gress RE. Immunosenescence: deficits in adaptive immunity in the elderly. *Tissue Antigens*. 2007; 70:179–189. [PubMed: 17661905]
- Hall-Stoodley L, Watts G, Crowther JE, Balagopal A, Torrelles JB, Robison-Cox J, Bargatze RF, Harmsen AG, Crouch EC, Schlesinger LS. *Mycobacterium tuberculosis* binding to human surfactant proteins A and D, fibronectin, and small airway epithelial cells under shear conditions. *Infect Immun*. 2006; 74:3587–3596. [PubMed: 16714591]
- Harada H, Fujita T, Miyamoto M, Kimura Y, Maruyama M, Furia A, Miyata T, Taniguchi T. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. *Cell*. 1989; 58:729–739. [PubMed: 2475256]
- Harris J, Keane J. How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clin Exp Immunol*. 2010; 161:1–9. [PubMed: 20491796]
- Henao-Tamayo M, Ordway DJ, Orme IM. Memory T cell subsets in tuberculosis: What should we be targeting? *Tuberculosis (Edinb)*. 2014; 94:455–461. [PubMed: 24993316]

- Henning LN, Azad AK, Parsa KV, Crowther JE, Tridandapani S, Schlesinger LS. Pulmonary surfactant protein A regulates TLR expression and activity in human macrophages. *J Immunol.* 2008; 180:7847–7858. [PubMed: 18523248]
- Houben RM, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. *PLoS Med.* 2016; 13:e1002152. [PubMed: 27780211]
- Johnson CM, Cooper AM, Frank AA, Orme IM. Adequate expression of protective immunity in the absence of granuloma formation in *Mycobacterium tuberculosis*-infected mice with a disruption in the intracellular adhesion molecule 1 gene. *Infection and immunity.* 1998; 66:1666–1670. [PubMed: 9529096]
- Juffermans NP, Florquin S, Camoglio L, Verbon A, Kolk AH, Speelman P, van Deventer SJ, Van Der PT. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J Infect Dis.* 2000; 182:902–908. [PubMed: 10950787]
- Kamath A, Woodworth JS, Behar SM. Antigen-specific CD8+ T cells and the development of central memory during *Mycobacterium tuberculosis* infection. *J Immunol.* 2006; 177:6361–6369. [PubMed: 17056567]
- Kang BK, Schlesinger LS. Characterization of mannose receptor-dependent phagocytosis mediated by *Mycobacterium tuberculosis* lipoarabinomannan. *Infect Immun.* 1998; 66:2769–2777. [PubMed: 9596746]
- Khan N, Shariff N, Cobbold M, Bruton R, Ainsworth JA, Sinclair AJ, Nayak L, Moss PA. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. *J Immunol.* 2002; 169:1984–1992. [PubMed: 12165524]
- Kirman JR, Henao-Tamayo MI, Agger EM. The Memory Immune Response to Tuberculosis. *Microbiol Spectr.* 2016; 4
- Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. Innate immune recognition of *Mycobacterium tuberculosis*. *Clinical & developmental immunology.* 2011; 2011:405310. [PubMed: 21603213]
- Krone CL, van de Groep K, Trzcinski K, Sanders EA, Bogaert D. Immunosenescence and pneumococcal disease: an imbalance in host-pathogen interactions. *Lancet Respir Med.* 2014; 2:141–153. [PubMed: 24503269]
- Ladel CH, Blum C, Dreher A, Reifenberg K, Kopf M, Kaufmann SH. Lethal tuberculosis in interleukin-6-deficient mutant mice. *Infect Immun.* 1997a; 65:4843–4849. [PubMed: 9353074]
- Ladel CH, Szalay G, Riedel D, Kaufmann SHE. Interleukin-12 secretion by *Mycobacterium tuberculosis*-infected macrophages. *Infect Immun.* 1997b; 65:1936–1938. [PubMed: 9125583]
- Lee SW, Oh DK, Lee SH, Kang HY, Lee CT, Yim JJ. Time interval to conversion of interferon-gamma release assay after exposure to tuberculosis. *The European respiratory journal.* 2011; 37:1447–1452. [PubMed: 21148232]
- LeMaout J, Messaoudi I, Manavalan JS, Potvin H, Nikolich-Zugich D, Dyall R, Szabo P, Weksler ME, Nikolich-Zugich J. Age-related dysregulation in CD8 T cell homeostasis: kinetics of a diversity loss. *J Immunol.* 2000; 165:2367–2373. [PubMed: 10946259]
- Leung GM, Hedley AJ, Ho LM, Chau P, Wong IO, Thach TQ, Ghani AC, Donnelly CA, Fraser C, Riley S, Ferguson NM, Anderson RM, Tsang T, Leung PY, Wong V, Chan JC, Tsui E, Lo SV, Lam TH. The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Annals of internal medicine.* 2004; 141:662–673. [PubMed: 15520422]
- Lin PL, Plessner HL, Voitenok NN, Flynn JL. Tumor necrosis factor and tuberculosis. *The journal of investigative dermatology Symposium proceedings.* 2007; 12:22–25. [PubMed: 17502865]
- Lin SI, Woollacott M. Association between sensorimotor function and functional and reactive balance control in the elderly. *Age Ageing.* 2005; 34:358–363. [PubMed: 15899911]
- Linehan E, Dombrowski Y, Snoddy R, Fallon PG, Kissenpfennig A, Fitzgerald DC. Aging impairs peritoneal but not bone marrow-derived macrophage phagocytosis. *Aging Cell.* 2014; 13:699–708. [PubMed: 24813244]
- Lloyd CM, Marsland BJ. Lung Homeostasis: Influence of Age, Microbes, and the Immune System. *Immunity.* 2017; 46:549–561. [PubMed: 28423336]

- Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013; 153:1194–1217. [PubMed: 23746838]
- Lovik M, North RJ. Effect of aging on antimicrobial immunity: old mice display a normal capacity for generating protective T cells and immunologic memory in response to infection with *Listeria monocytogenes*. *J Immunol*. 1985; 135:3479–3486. [PubMed: 3876387]
- Lyons CR, Lovchik J, Hutt J, Lipscomb MF, Wang E, Heninger S, Berliba L, Garrison K. Murine model of pulmonary anthrax: kinetics of dissemination, histopathology, and mouse strain susceptibility. *Infection and immunity*. 2004; 72:4801–4809. [PubMed: 15271942]
- Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: human memory T-cell subsets. *European journal of immunology*. 2013; 43:2797–2809. [PubMed: 24258910]
- McDonough KA, Kress Y, Bloom BR. Pathogenesis of tuberculosis: Interaction of *Mycobacterium tuberculosis* with macrophages. *Infect Immun*. 1993; 61:2763–2773. [PubMed: 8514378]
- Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ. Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J Immunol*. 1999; 163:3920–3927. [PubMed: 10490993]
- Medina E, North RJ. Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and Nramp1 genotype. *Immunology*. 1998; 93:270–274. [PubMed: 9616378]
- Mehta JB, Dutt AK. Tuberculosis in the elderly. *Infect Med*. 1995; 12:40–46.
- Mendez-Samperio P. Expression and regulation of chemokines in mycobacterial infection. *J Infect*. 2008; 57:374–384. [PubMed: 18838171]
- Messaoudi I, Lemaout J, Guevara-Patino JA, Metzner BM, Nikolich-Zugich J. Age-related CD8 T cell clonal expansions constrict CD8 T cell repertoire and have the potential to impair immune defense. *The Journal of experimental medicine*. 2004; 200:1347–1358. [PubMed: 15545358]
- Meyer KC. The role of immunity in susceptibility to respiratory infection in the aging lung. *Respir Physiol*. 2001; 128:23–31. [PubMed: 11535259]
- Moliva JJ, Hossfeld AP, Canan CH, Dwivedi V, Wewers MD, Beamer G, Turner J, Torrelles JB. Exposure to human alveolar lining fluid enhances *Mycobacterium bovis* BCG vaccine efficacy against *Mycobacterium tuberculosis* infection in a CD8+ T-cell-dependent manner. *Mucosal Immunol*. 2017
- Moliva JJ, Rajaram MV, Sidiki S, Sasindran SJ, Guirado E, Pan XJ, Wang SH, Ross P Jr, Lafuse WP, Schlesinger LS, Turner J, Torrelles JB. Molecular composition of the alveolar lining fluid in the aging lung. *Age (Dordr)*. 2014; 36:9633. [PubMed: 24584696]
- Moreira-Teixeira L, Redford PS, Stavropoulos E, Ghilardi N, Maynard CL, Weaver CT, Freitas do Rosario AP, Wu X, Langhorne J, O'Garra A. T Cell-Derived IL-10 Impairs Host Resistance to *Mycobacterium tuberculosis* Infection. *J Immunol*. 2017; 199:613–623. [PubMed: 28584007]
- Negin J, Abimbola S, Marais BJ. Tuberculosis among older adults—time to take notice. *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*. 2015; 32:135–137. [PubMed: 25809769]
- Niccoli T, Partridge L. Ageing as a risk factor for disease. *Current biology: CB*. 2012; 22:R741–752. [PubMed: 22975005]
- Notter, RH. Lung surfactants: Basic science and clinical applications. New York: Marcel Dekker; 2000.
- Orme I. Mechanisms underlying the increased susceptibility of aged mice to tuberculosis. *Nutr Rev*. 1995; 53(Suppl):S35–S40. [PubMed: 7644152]
- Orme IM. Aging and immunity to tuberculosis: increased susceptibility of old mice reflects a decreased capacity to generate mediator T lymphocytes. *J Immunol*. 1987; 138:4414–4418. [PubMed: 3495592]
- Orme IM. A mouse model of the recrudescence of latent tuberculosis in the elderly. *Am Rev Respir Dis*. 1988; 137:716–718. [PubMed: 3345049]
- Orme IM, Basaraba RJ. The formation of the granuloma in tuberculosis infection. *Semin Immunol*. 2014; 26:601–609. [PubMed: 25453231]

- Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. *Nat Immunol.* 2015; 16:57–63. [PubMed: 25521685]
- Paton NI, Ng YM. Body composition studies in patients with wasting associated with tuberculosis. *Nutrition* (Burbank, Los Angeles County, Calif). 2006; 22:245–251.
- Philips JA, Ernst JD. Tuberculosis pathogenesis and immunity. *Annu Rev Pathol.* 2012; 7:353–384. [PubMed: 22054143]
- Posnett DN, Sinha R, Kabak S, Russo C. Clonal populations of T cells in normal elderly humans: the T cell equivalent to “benign monoclonal gammopathy”. *The Journal of experimental medicine.* 1994; 179:609–618. [PubMed: 8294871]
- Quesniaux VF, Jacobs M, Allie N, Grivennikov S, Nedospasov SA, Garcia I, Olleros ML, Shebzukhov Y, Kuprash D, Vasseur V, Rose S, Court N, Vacher R, Ryffel B. TNF in host resistance to tuberculosis infection. *Current directions in autoimmunity.* 2010; 11:157–179. [PubMed: 20173394]
- Rajagopalan S. Tuberculosis and aging: a global health problem. *Clin Infect Dis.* 2001; 33:1034–1039. [PubMed: 11528577]
- Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. *Nat Rev Immunol.* 2012; 12:352–366. [PubMed: 22517424]
- Redford PS, Boonstra A, Read S, Pitt J, Graham C, Stavropoulos E, Bancroft GJ, O’Garra A. Enhanced protection to *Mycobacterium tuberculosis* infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. *Eur J Immunol.* 2010; 40:2200–2210. [PubMed: 20518032]
- Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol.* 2002; 169:4697–4701. [PubMed: 12391175]
- Reynolds HY. Lung inflammation: normal host defense or a complication of some diseases? *Annual review of medicine.* 1987; 38:295–323.
- Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent *M.tb*. *Tubercle Lung Dis.* 1997; 78:57–66.
- Robinson RT, Orme IM, Cooper AM. The onset of adaptive immunity in the mouse model of tuberculosis and the factors that compromise its expression. *Immunol Rev.* 2015; 264:46–59. [PubMed: 25703551]
- Rottinghaus EK, Vesosky B, Turner J. Interleukin-12 is sufficient to promote antigen-independent interferon-gamma production by CD8 T cells in old mice. *Immunology.* 2009:e679–e690. 128. [PubMed: 19740329]
- Rottinghaus EK, Vesosky B, Turner J. TLR-2 independent recognition of *Mycobacterium tuberculosis* by CD11c+ pulmonary cells from old mice. *Mech Ageing Dev.* 2010; 131:405–414. [PubMed: 20566357]
- Sanchez D, Rojas M, Hernandez I, Radzioch D, Garcia LF, Barrera LF. Role of TLR2- and TLR4-mediated signaling in *Mycobacterium tuberculosis*-induced macrophage death. *Cell Immunol.* 2010; 260:128–136. [PubMed: 19919859]
- Sasindran J, Torrelles J. *Mycobacterium tuberculosis* infection and inflammation: What is beneficial for the host and for the bacterium? *Frontiers in Microbiology.* 2011; 2:1–16. [PubMed: 21716958]
- Saule P, Trauet J, Dutriez V, Lekeux V, Dessaint JP, Labalette M. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. *Mechanisms of ageing and development.* 2006; 127:274–281. [PubMed: 16352331]
- Schlesinger, LS., Azad, AK., Torrelles, JB., Roberts, E., Vergne, I., Deretic, V. Determinants of phagocytosis, phagosome biogenesis and autophagy for *Mycobacterium tuberculosis*. In: Kaufmann, SHE., Britton, WJ., editors. *Handbook of Tuberculosis Immunology and Cell Biology.* Weinheim, Germany: Wiley-VCH Verlag GmbH&Co. KGaA; 2008.
- Scordo J, Arcos J, HV K, Sasindran J, Diangelo L, Lee E, Wewers M, Wang S, Balada-Llasat J, Torrelles J. *Mycobacterium tuberculosis* cell wall fragments released upon bacterial contact with the human lung mucosa alter the neutrophil response to infection. *Front Immunol.* 2017

- Serbina NV, Flynn JL. CD8(+) T cells participate in the memory immune response to *Mycobacterium tuberculosis*. *Infection and immunity*. 2001; 69:4320–4328. [PubMed: 11401969]
- Shaw AC, Panda A, Joshi SR, Qian F, Allore HG, Montgomery RR. Dysregulation of human Toll-like receptor function in aging. *Ageing Res Rev*. 2011; 10:346–353. [PubMed: 21074638]
- Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, Enninga J. Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. *PLoS Pathog*. 2012; 8:e1002507. [PubMed: 22319448]
- Smith CM, Proulx MK, Olive AJ, Laddy D, Mishra BB, Moss C, Gutierrez NM, Bellerose MM, Barreira-Silva P, Phuah JY, Baker RE, Behar SM, Kornfeld H, Evans TG, Beamer G, Sasseti CM. Tuberculosis Susceptibility and Vaccine Protection Are Independently Controlled by Host Genotype. *MBio*. 2016; 7
- Smithey MJ, Li G, Venturi V, Davenport MP, Nikolich-Zugich J. Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. *J Immunol*. 2012; 189:5356–5366. [PubMed: 23087407]
- Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B. Regulation of MHC class II expression by interferon- γ mediated by the transactivator gene CIITA. *Science*. 1994; 265:106–109. [PubMed: 8016643]
- Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol*. 2005; 175:342–349. [PubMed: 15972667]
- Torrelles JB, Azad AK, Henning LN, Carlson TK, Schlesinger LS. Role of C-type lectins in mycobacterial infections. *Curr Drug Targets*. 2008; 9:102–112. [PubMed: 18288961]
- Torrelles JB, Schlesinger LS. Integrating lung physiology, immunology, and tuberculosis. *Trends Microbiol*. 2017
- Trotta R, Ciarlariello D, Dal Col J, Allard J 2nd, Neviani P, Santhanam R, Mao H, Becknell B, Yu J, Ferketich AK, Thomas B, Modi A, Blaser BW, Perrotti D, Caligiuri MA. The PP2A inhibitor SET regulates natural killer cell IFN- γ production. *The Journal of experimental medicine*. 2007; 204:2397–2405. [PubMed: 17875674]
- Turnbull IR, Clark AT, Stromberg PE, Dixon DJ, Woolsey CA, Davis CG, Hotchkiss RS, Buchman TG, Coopersmith CM. Effects of aging on the immunopathologic response to sepsis. *Crit Care Med*. 2009; 37:1018–1023. [PubMed: 19237912]
- Turner J, Frank AA, Orme IM. Old mice express a transient early resistance to pulmonary tuberculosis that is mediated by CD8 T cells. *Infect Immun*. 2002; 70:4628–4637. [PubMed: 12117976]
- Turner J, Orme IM. The expression of early resistance to an infection with *Mycobacterium tuberculosis* by old mice is dependent on IFN type II (IFN- γ) but not IFN type I. *Mech Ageing Dev*. 2004; 125:1–9. [PubMed: 14706232]
- Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci USA*. 1999; 96:14459–14463. [PubMed: 10588727]
- van der Wel N, Hava D, Houben D, Fluittsma D, van ZM, Pierson J, Brenner M, Peters PJ. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. *Cell*. 2007; 129:1287–1298. [PubMed: 17604718]
- Vesosky B, Flaherty DK, Rottinghaus EK, Beamer GL, Turner J. Age dependent increase in early resistance of mice to *Mycobacterium tuberculosis* is associated with an increase in CD8 T cells that are capable of antigen independent IFN- γ production. *Exp Gerontol*. 2006a; 41:1185–1194. [PubMed: 17029663]
- Vesosky B, Flaherty DK, Turner J. Th1 cytokines facilitate CD8-T-cell-mediated early resistance to infection with *Mycobacterium tuberculosis* in old mice. *Infect Immun*. 2006b; 74:3314–3324. [PubMed: 16714559]
- Vesosky B, Rottinghaus EK, Davis C, Turner J. CD8 T Cells in old mice contribute to the innate immune response to *Mycobacterium tuberculosis* via interleukin-12p70-dependent and antigen-independent production of gamma interferon. *Infect Immun*. 2009; 77:3355–3363. [PubMed: 19470747]

- Vesosky B, Turner J. The influence of age on immunity to infection with *Mycobacterium tuberculosis*. *Immunol Rev*. 2005; 205:229–243. [PubMed: 15882357]
- Weiskopf D, Weinberger B, Grubeck-Loebenstien B. The aging of the immune system. *Transplant international: official journal of the European Society for Organ Transplantation*. 2009; 22:1041–1050. [PubMed: 19624493]
- WHO. Global Tuberculosis Report 2016. 2017
- Winslow GM, Cooper A, Reiley W, Chatterjee M, Woodland DL. Early T-cell responses in tuberculosis immunity. *Immunol Rev*. 2008; 225:284–299. [PubMed: 18837789]
- Woodworth JS, Behar SM. *Mycobacterium tuberculosis*-specific CD8+ T cells and their role in immunity. *Crit Rev Immunol*. 2006; 26:317–352. [PubMed: 17073557]
- Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *The Journal of experimental medicine*. 2008; 205:711–723. [PubMed: 18332179]
- Yamada H, Mizumo S, Horai R, Iwakura Y, Sugawara I. Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. *Lab Invest*. 2000; 80:759–767. [PubMed: 10830786]
- Zevallos M, Justman JE. Tuberculosis in the elderly. *Clinics in geriatric medicine*. 2003; 19:121–138. [PubMed: 12735118]
- Zumla A, Raviglione M, Hafner R, Von Reyn CF. Tuberculosis. *N Engl J Med*. 2013; 368:745–755. [PubMed: 23425167]

Table1

Changes in the molecular, innate, and functional lung environment with age

Component	Change	Reference
<i>Overall lung Inflammation</i>	Increased presence	(Canan et al. 2014)
<i>Early response to M.tb</i>	Enhanced	(Turner et al. 2002)
<i>Late/long-term response to M.tb</i>	Diminished	(Orme 1987)
<i>Lung microenvironment/ALF fluid</i>		(Moliva et al. 2014)
-TNF, IL-12, IL-1 β	Increased amounts	
-SP-A, SP-D	Increased amounts	
-Complement components (C3b)	Increased amounts	
-Hydrolytic enzymes(hydrolases)	Decreased amounts	
- Overall oxidative status	Increased	
-Oxidized surfactant lipids	Increased amounts	
<i>CD11c⁺ cells in lung</i>		(Rottinghaus et al. 2010)
TLR2 expression	Increased	
Cytokine secretion after <i>M.tb</i> stimulation	Total amounts unchanged	
TLR2 reliance for cytokine secretion	No dependence	
TLR4/9 reliance for cytokine secretion	Partial dependence	
<i>Pulmonary macrophages</i>		
-Transcription factors: IRGM-1, CIITA, IRF-1	Increased amounts	(Canan et al. 2014)
<i>After M.tb infection</i>		(Canan et al. 2014)
-Total infected cells	Increased numbers	
-P+L fusion	Increased	
<i>Memory CD8 T cells</i>		
-Memory CD8 T cells in lung	Increased amounts Capable	(Vesosky et al. 2006b) (Vesosky et al. 2006a)
-IFN- γ secretion in response to IL-12 alone		
-Cytokine receptors (IL-18, IL-2, IL-12, IFN- γ)	Increased expression	
-pSTAT4 phosphorylation and expression in response to IL-12	Increased	(Rottinghaus et al. 2009)
-SET expression	Increased	(Vesosky et al. 2009)