Don't cough on me!
Airborne Transmission of Disease

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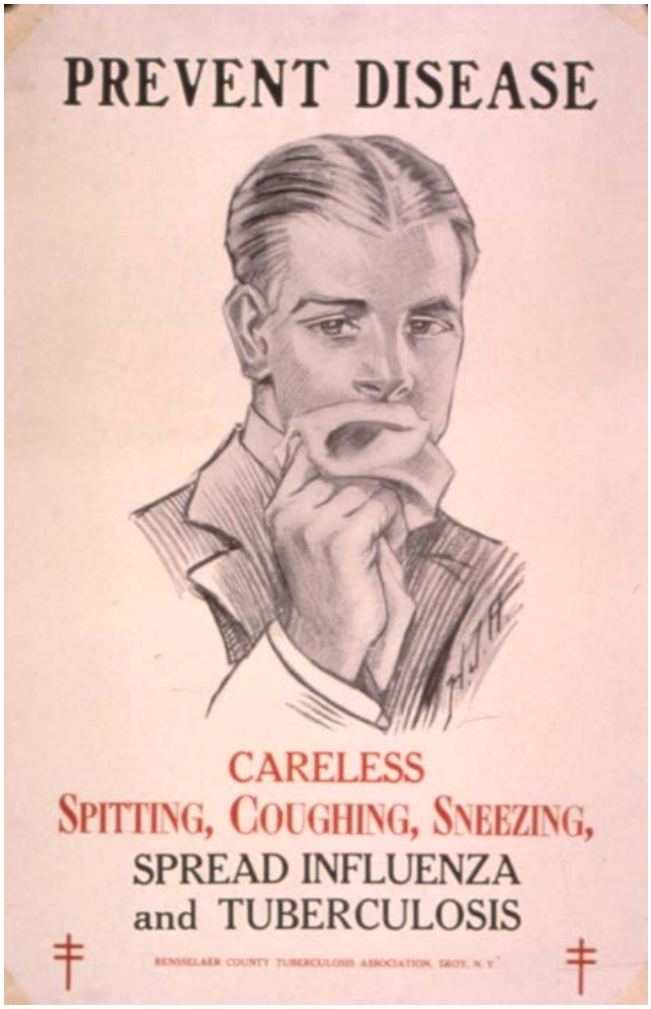

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Academic Interests:
My research involves *Mycobacterium tuberculosis* pathogenesis with a focus on mucosal immunology and innate immunity. I strive to connect in vitro and animal studies to human tissues and models thereby applying the work done in my lab to human disease. Ongoing projects include characterization of microfold cell-mediated entry mechanisms of *Mycobacterium tuberculosis*, discovery of novel enzymes and virulence factors of *Mycobacterium tuberculosis*, evaluation of targeting autophagy as novel therapeutics for *Mycobacterium tuberculosis* infection, and elucidation of the mechanisms of cough induction by *Mycobacterium tuberculosis* and the role of cough in transmission.

Purpose and overview:
The purpose of this Grand Rounds is to review the aerobiology of infectious diseases in general with an ultimate emphasis on transmission of disease via cough and specifically focus on possible mechanisms responsible for cough in the transmission of *Mycobacterium tuberculosis*. Routes of aerosolized infection transmission, details regarding the cough reflex, and proposed molecular mechanisms of cough induction by respiratory diseases, namely pulmonary tuberculosis, will be discussed.

Educational objectives:
1. Understand the role of aerobiology in the spread of disease
2. Identify primary mechanisms of transmission for common aerosolized diseases and sources of aerosolized infection
3. Appreciate that the cough reflex is a complicated event that involves coordinated activity between the nervous and musculoskeletal systems
4. Be aware of the early studies elucidating that the transmission of *Mycobacterium tuberculosis* is via an airborne route
5. Recognize that there is limited data on the molecular mechanisms of cough induction by respiratory infection

Introduction

Infections are set apart from other diseases by a variety of characteristics including their transmissibility. While the spread of infectious diseases can result in sweeping global effects exemplified by the bubonic-pneumonic plague of the 14th century, the 1918 influenza pandemic, and the current epidemic of HIV/AIDS, there is actually little variety in the mechanisms of transmission: namely via direct inoculation, waterborne transmission, and airborne transmission (Fauci and Morens, 2012). Because of this and the fact that most infectious diseases are caused by a single agent (unlike many chronic and lifestyle-associated diseases such as hypertension and diabetes that result from multiple interacting factors), infections have the potential to be well-controlled via medical and public health interventions once the pathogen and the route of transmission are effectively identified. As history has shown, however, identification of the offending infectious agent as well as the mechanism of its transmission are not always simple to understand, making infectious diseases a continual threat to global health.

Before the germ theory of disease was accepted in the late 1800s, the prevailing dogma in the medical profession regarding disease transmission was the miasma theory which held that nearly all diseases were caused by "inhaling air that was infected through exposure to corrupting matter" (Halliday, 2001). Indeed, official investigation of the cholera epidemic in 1849 by the General Board of Health in England concluded that transmission was airborne due to "nocturnal vapors emanating from the Thames River" until John Snow's independent investigation of the epidemic ultimately revealed that cholera was, in fact, waterborne (Roy and Milton, 2004). Moreover, prior to publications by Joseph Lister regarding the importance of antiseptic technique in reduction of surgical site infections, many believed postoperative infections were due entirely to exposure of the open wounds to "bad air" (Lister, 1867). While subsequent research has clearly shown that infections are transmitted by numerous routes, the airborne mechanism of transmission continues to play an important role in the propagation of many infectious diseases.

Three key phases in a pathogen's life cycle dictate its ability to propagate: (1) invasion (2) survival and proliferation and (3) escape beyond the host to infect naïve individuals. Many pathogenic bacteria interact with hosts by secreting virulence molecules like proteins and lipids. A classic example is the production of cholera toxin by *Vibrio cholera*, which enhances the pathogen's spread from person-to-person by promoting profuse watery diarrhea.

However, the exact mechanisms by which the vast majority of infectious organisms facilitate their own transmission is poorly understood. In particular, how pulmonary pathogens enhance their own transmission by inducing infected hosts to produce airborne particles (such as through stimulation of cough) is not well known. In this Grand Rounds the aerobiology of infectious diseases in general will be reviewed with an ultimate emphasis on transmission of disease via cough and specific focus on possible mechanisms responsible for cough in the transmission of *Mycobacterium tuberculosis*.

Aerobiology of disease

Aerobiology is the study of the processes involved in the movement of microorganisms, including pathogens, from one geographic location to another in the environment (Fernstrom and Goldblatt, 2013). Seminal studies conducted in the 1930s by William Firth Wells first elucidated that spread of microorganisms in the air highly depends upon the settling of expelled particles and that settling of such particles is intimately associated with particle size, time, and evaporation (Wells et al., 1939). From studies done by Wells and others, it was further recognized that aerosolized spread of disease occurs via droplet and airborne mechanisms. Droplet transmission is transmission due to expelled particles with a high propensity to settle quickly to the ground, typically within 1 meter of the site of generation, due to size. As such, infection via droplet transmission relies on close proximity between infected and susceptible hosts. Settled droplets are also able to facilitate fomite transmission of infection. In comparison, airborne transmission involves relatively smaller particles that can remain suspended in the air for prolonged periods of time thereby allowing for the potential exposure and subsequent infection of a large number of susceptible hosts at a relatively greater distance away from the source (Gralton et al., 2011). Many studies have shown that particles can actually remain airborne for more than a week and can be found at over 20 meters from the originating source depending on various environmental factors (including humidity, temperature, and pressure differentials) (Fernstrom and Goldblatt, 2013), which proves critical in disease transmission due to airborne bacterial, viral, and fungal particles. Put simply, large particles “drop” as droplets out of the air whereas small particles remain airborne.

Per the World Health Organization, organisms less than or equal to 5 μm can cause airborne transmission and organisms greater than 5 μm result in droplet transmission (Table 1). However, many factors complicate the

Table 1: Primary Mechanisms of Transmission for Common Aerosolized Diseases

Transmitted via droplets	Transmitted via airborne particles
Bordetella pertussis	*Mycobacterium tuberculosis*
Influenza viruses	Influenza viruses
Adenoviruses	Varicella Zoster virus
Rhinoviruses	Rhinoviruses
Mycoplasma pneumoniae	Rubeola virus
SARS-associated coronavirus	Variola viruses
Streptococcus pyogenes	Norovirus
Neisseria meningitidis	Rotavirus
Respiratory syncytial virus (RSV)	*Aspergillus* species

simplistic definition based on size alone, such as the fact that even large droplets can desiccate rapidly and become smaller particles, which then can subsequently become airborne. In addition, droplet and airborne transmission are known to not be mutually exclusive in that particles carrying infectious microorganisms do not always spread exclusively via either the airborne or droplet route but rather via both methods simultaneously regardless of their origin (Fernstrom and Goldblatt, 2013). Nonetheless, although size limits for droplet and airborne dissemination are not always completely clear, the fact that particle size is extremely important in the aerobiology of disease is well documented. The ability of an infectious microorganism to veritably cause infection depends on the concentration of the microorganism, the infectious dose (which is also dependent upon host factors), and the virulence of the pathogen. Generally speaking, microorganisms spread via droplets (relatively larger particles) tend to require a larger inoculum for transmission, whereas those microorganisms spread via the airborne route (relatively smaller particles), need only a very small amount of pathogen to transmit disease. For example, Influenza A has been shown to transmit via droplet and airborne means, requiring a very small dose to cause infection, and only a few cells of *Mycobacterium tuberculosis* are required to infect a susceptible host (Fernstrom and Goldblatt, 2013).

Although airborne disease spread depends on inherent physical variables of the actual microorganism particle, environmental factors, namely humidity and temperature, dramatically influence transmission efficacy. In most cases, low temperature and humidity allows for greater survival of viruses whereas low temperature but high humidity increases the likelihood of bacteria

survival. For example, influenza has been shown to be highly transmissible via the airborne route under cold and dry conditions (Lowen et al., 2007) and *Mycoplasma pneumoniae* has been shown to be most viable in cool and humid conditions (Wright et al., 1969). There is a relative paucity of data regarding the effects of temperature and relative humidity on the airborne transmission of fungi, but various trends in seasonal variations of environmental molds suggest that these factors play a role in disease spread. However, fungi and their spores seem to be more resilient to dehydration and rehydration than viral and bacterial pathogens (Fernstrom and Goldblatt, 2013).

Sources of Aerosolized Infection

Whether an infection results from aerosolized microorganisms depends on many variables including the presence of a pathogen and a variety of host factors. However, there are several well-known primary sources of potentially infectious particles (Table 2). While a single sneeze can generate as much as 40,000 large droplet particles, most of these particles will desiccate immediately into smaller droplets. Moreover, although one sneeze produces more total potentially infectious particles than a single cough, disease processes causing high frequency of cough (such as pulmonary tuberculosis) allow for an increased risk of disease transmission via coughing as opposed to a more efficient mechanism (Buckland and Tyrrell, 1964).

Table 2: Microorganisms Released During Various Activities

Activity	Approximate particle count
Sneezing	40,000 per sneeze
Coughing	710 per cough
Talking	36 per 100 words
Vomiting	1,000 per event
Bowel evacuation	20,000 per event

(Adapted from (Fernstrom and Goldblatt, 2013)

Aerosolized infections can also be spread from the environment (as is the case for many endemic fungi including *Histoplasmosis* species and *Aspergillus* species) and many infection prevention and control measures focus upon proper ventilation and air filtration. However, further discussion of such mechanisms of transmission are out of the scope of this Grand Rounds, which is geared more toward better understanding human-to-human transmission of disease.

The cough reflex

Although aerosolization of infection can occur via various mechanisms as mentioned above, it is known that certain infections lead to specific symptoms that may result in their effective transmission from an infected individual to an uninfected individual. For example, *Bordetella pertussis* induces "whooping cough" and active pulmonary tuberculosis often causes hemoptysis, making cough a likely primary means of transmission for both of these diseases. Although on the surface a cough may seem like a simple response of the pulmonary system to noxious stimuli, the actual fundamental mechanisms that occur at the molecular level to generate a cough are quite complex.

The cough reflex is a complicated event that involves coordinated activity between the nervous and musculoskeletal systems. This reflex, which may have evolved as a pulmonary defense against aspiration of food and gastric contents or inhalation of irritants and infectious particles, is a highly-organized, neuromuscular response that is conserved across mammalian species (Brooks, 2011; Canning, 2008; Canning, 2010) and can be initiated by both chemosensory and mechanosensory pulmonary neurons (Mazzone and Undem, 2016). Cough is triggered by pain or irritation-sensing neurons

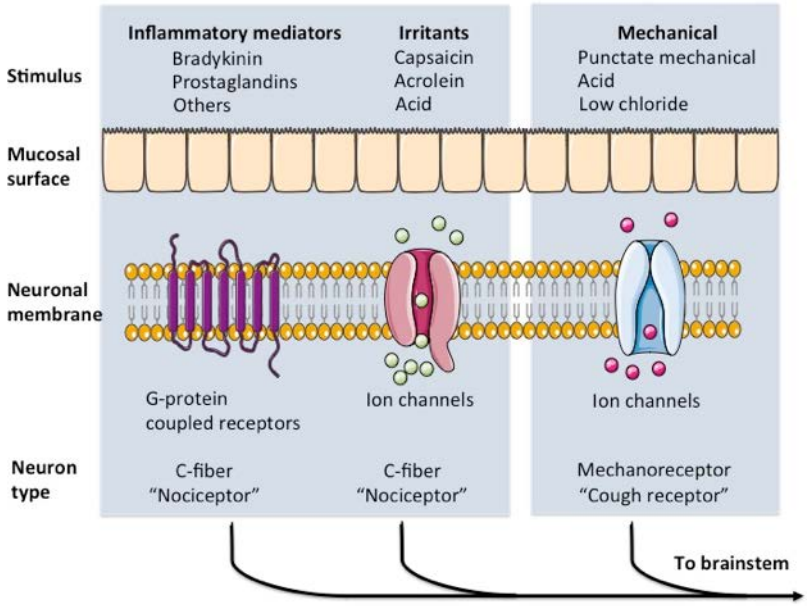

Figure 1: Peripheral mechanisms of cough. Inflammatory mediators and irritants stimulate C-fiber neurons, while mechanical stimuli stimulate mechaoreceptors.

(also called nociceptive neurons) being activated by various particulate or chemical molecules. This activation initiates a signaling cascade ultimately

resulting in cough (Figure 1). Respiratory nociceptive neurons innervate the airway and lungs of humans and most mammals and as such are poised to respond to noxious molecules to protect the lung from damage. Afferent chemosensory neurons (C-fibers) respond to a variety of exogenous noxious molecules like capsaicin and particulates, as well as endogenous molecules like acid, ATP, prostaglandins and bradykinin. Such C-fiber neurons have been directly visualized in whole mount biopsies of mouse, rat, guinea pig and human lungs (De Proost et al., 2007a; De Proost et al., 2007b; Pintelon et al., 2007), where their peripheral terminals can be found both in the airways and lung parenchyma (Mazzone and Undem, 2016). While initiating a pulmonary response to respiratory infection is considered a major function of the cough reflex, to date there is limited data on the molecular mechanisms of cough induction by respiratory infection. In particular, it is unknown if airway afferent neurons are directly stimulated by virulence components from pathogens. For example, though *Bordetella pertussis* provokes the characteristic "whooping cough" in children and produces many virulence factors, none of the known protein toxins, including the well-studied pertussis toxin, actually induce cough (Cherry, 2013; Hewlett et al., 2014).

Airborne transmission of tuberculosis

Recognized as the deadliest infectious disease of the 19th century (Fauci and Morens, 2012), *Mycobacterium tuberculosis* remains a global health threat causing 8 million cases of tuberculosis with 1.3 million deaths worldwide annually and an estimated prevalence of 2 billion latently infected individuals. Robert Koch's description of *Mycobacterium tuberculosis* as the etiologic agent of tuberculosis in 1882 was a landmark discovery not only in the microbiology field but also for public health. However, following his discovery there remained significant debate over the means of *Mycobacterium tuberculosis* transmission and various routes were proposed including airborne, oral, contact, and vertical transmission.

In addition to demonstrating that experimental inoculation of *Mycobacterium tuberculosis*-infected material into naïve animals could recapitulate tuberculosis disease (one of "Koch's postulates"), Koch introduced the guinea pig as an animal model for infectious disease research and made some preliminary observations that naïve guinea pigs housed in the same room as infected animals developed "spontaneous" (i.e. without being directly inoculated) pulmonary tuberculosis (Koch, 1912). Several decades later, David Perla confirmed that a cohort of guinea pigs housed in separate cages but exposed to other tuberculous animals including infected guinea pigs and

rabbits developed bronchogenic tuberculosis. Moreover, he directly demonstrated that cohousing *Mycobacterium tuberculosis*-infected with naïve guinea pigs resulted in the development of gastrointestinal tuberculosis in between 30-60% of the naïve guinea pig population via fecal-oral infection (Perla, 1927a; Perla, 1927b). To eliminate the enteric route as a portal of entry, Max Lurie performed similar cohousing experiments with a modified system for housing the guinea pigs. By placing the animals in cages with wire floors that greatly reduced exposure to contaminated feces, he detected an incidence of tuberculosis in naïve contact of 21% through primarily a bronchial route (Lurie, 1930b; Lurie, 1930c). In parallel experiments, Lurie also observed an incidence rate of 15% in guinea pigs housed in the same room as infected animals but in separate cages, further illustrating the likely airborne route of transmission (Lurie, 1930a). Interestingly, the incidence of spontaneous tuberculosis rose significantly over time, and of those animals exposed for nearly 2 years, 35% developed pathologically evident tuberculosis (Lurie, 1930a). In all of these studies, determining the true transmission rate was hampered by the reliance on autopsy for diagnosis and the lack of immunologic methods such as the tuberculin skin test or an interferon-gamma release assay for detecting infection. Thus, while these seminal studies identified the airborne route in guinea pig infection as the most likely to recapitulate human disease caused by tuberculosis, the actual rate of transmission within guinea pigs during "natural exposure" was likely significantly higher than reported as some exposed animals undoubtedly developed latent infection without manifesting obvious signs of disease. Indeed, in a recent study using the methods of Richard Riley (Riley et al., 1957), 75% of guinea pigs exposed to air from a tuberculosis ward in South Africa developed a positive tuberculin skin test suggesting exposure and infection, but only 12% developed histopathologic evidence of tuberculosis disease at the time of autopsy (Dharmadhikari et al., 2011).

Cough induction by *Mycobacterium tuberculosis*

Cough is a primary symptom of active pulmonary tuberculosis. This symptom is instrumental in disease transmission, as the forceful aerosol generated by a tuberculosis-infected individual during a cough contains numerous infectious *Mycobacterium tuberculosis* particles that can then be inhaled by an uninfected person, thus resulting in a newly infected individual (Fennelly and Jones-Lopez, 2015; Fennelly et al., 2004; Jones-Lopez et al., 2015). Given the vital importance of cough in the transmission of tuberculosis, it is surprising how little is known about the biology of infection-mediated cough in general and tuberculosis-related cough in particular (Turner and Bothamley, 2015).

Importantly, seminal work performed at Parkland Hospital 50 years ago by Loudon and Brown using special cough monitoring equipment (Loudon and Romans, 1967) demonstrated that nighttime cough was a frequent occurrence in pulmonary tuberculosis that was associated with severity of disease (Loudon and Brown, 1967). They went on to show that individuals with more severe disease on chest x-ray coughed more, and were more likely to have close contacts that were PPD positive (Loudon and Spohn, 1969). In addition, they demonstrated that cough frequency, which averaged about 110 coughs over an 8 hour period on admission for active tuberculosis, declined rapidly after the onset of therapy (Loudon and Spohn, 1969). This work was recently confirmed in a study performed in Peru (Proano et al., 2017). Important observations from this study are that increased cough frequency is associated with sputum culture positivity by the MODS assay, as well as decreased time to positivity, a surrogate for bacterial load, and that cough frequency decreases rapidly after therapy initiation, consistent with prior observations (Proano et al., 2017). Finally, a recent report found that individuals with smear positive tuberculosis had more coughs than those with smear negative disease, though there was significant variation (Turner et al., 2014). Both groups had significantly more coughs than latent tuberculosis controls. Taken together, these studies in humans with tuberculosis suggest that there is a correlation

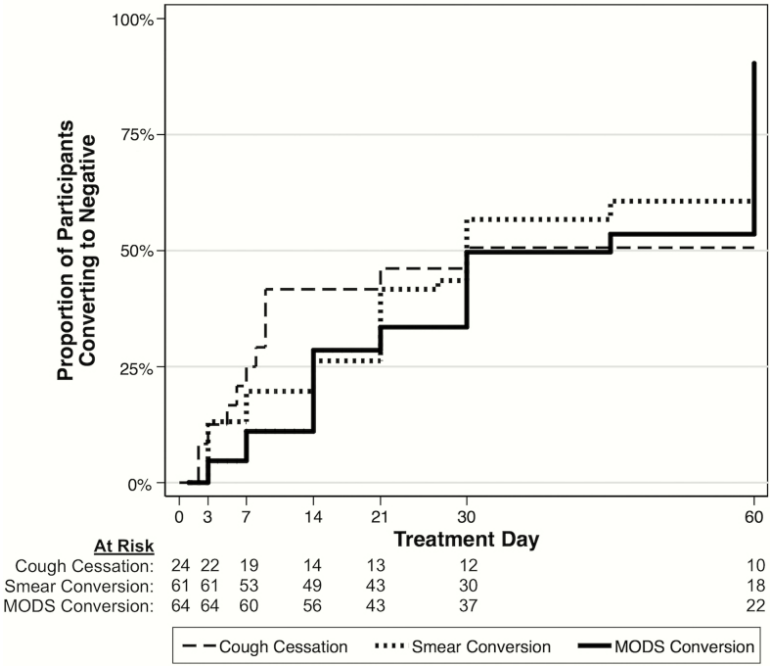

Fig. 2 Kaplan-Meier curves for time to coughing cessation and microbiological conversion in study group. Reproduced from (Proano et al., 2017) under a Creative Commons Attribution License.

between extent and severity of disease, which correlates with bacterial burden, and cough frequency.

The clinical and epidemiologic data regarding the relationship of disease severity (and bacterial burden) to cough frequency and rates of transmission raises an important question, namely, what are the molecular mechanisms of cough initiation in the setting of tuberculosis? Cough may be a natural consequence of lung inflammation and host production of prostaglandins, bradykinin and other inflammatory mediators that activate afferent neuronal C-fibers in the lung mucosa (Polverino et al., 2012). In addition, cavity or cyst formation itself might induce mechanical activation of either rapidly adapting receptors or slowly adapting stretch receptors that can sensitize the lungs to cough triggers (Mazzone, 2005; Mazzone and Undem, 2016). Conversely, cough itself, perhaps triggered by secreted mycobacterial factors, could lead to Mtb aerosolization and/or cavity formation. In this model, a granuloma or region of caseous necrotic pneumonia could be induced to form a cavity by very high mechanical forces (intrathoracic pressures as high as 300 mm Hg and expiratory velocities as high as 800 km/h) generated by a strenuous cough (Polverino et al., 2012), forcing weakened extracellular matrix and elastic tissue (Elkington et al., 2011) to stretch into a cavity. Thus, while cough may be a major route of aerosolization and spread, it may precede and/or overlap with the cavitary disease stage. Taken together, the current paradigm is that cough in tuberculosis, and likely many other infections, is a consequence of airway damage or irritation that leads to activation of nociceptive neurons (Turner and Bothamley, 2015). However, appealing as they may be, direct evidence for these models is lacking.

As was outlined above, the cough reflex is a complicated event that is triggered by nervous system activation. Of note, a variety of bacterial, protozoan, fungal and viral pathogens are known to engage the nervous system during their life cycles. While these interactions often involve direct infection of neuronal or accessory cells, recent studies have revealed that pathogens can release molecules to directly target nerves (Lim et al., 2016). For example, *Staphylococcus aureus* releases formyl peptides and alpha-hemolysin to activate dermal nociceptors resulting in pain and inflammation (Chiu et al., 2013). Moreover, an *E. coli* lysate activates nociceptive neurons in the colon (Ochoa-Cortes et al., 2010), likely due to neuronal excitation by lipopolysaccharide via the transient receptor potential (TRP) family member TRPA1 (Meseguer et al., 2014). In addition, *Mycobacterium ulcerans* produces mycolactone, a macrocyclic polyketide, that not only modulates the

immune system (Hall et al., 2014) but also directly anesthetizes peripheral nerves via the angiotensin 2 receptor (Marion et al., 2014). *Mycobacterium tuberculosis* does not generate mycolactone, but instead produces a diverse repertoire of lipid-based polyketide molecules (Quadri, 2014) as well as many other bioactive lipid and small molecules within the complex cell wall of *Mycobacterium tuberculosis* (Bailo et al., 2015; Jankute et al., 2015) whose functions are slowly being elucidated (Figure 3). Whether *Mycobacterium tuberculosis* directly triggers nociceptive neurons thereby inducing cough to facilitate its own transmission has not been explored.

Thus, because nociceptive neurons mediate cough and some bacteria, including mycobacteria, produce complex molecules that activate neurons, we hypothesized that *Mycobacterium tuberculosis* may produce organic-phase molecules that trigger nociceptive neurons to activate the cough response, thereby facilitating disease transmission (Figure 4). Indeed, we identified two organic-phase virulence lipids produced by *Mycobacterium tuberculosis* that

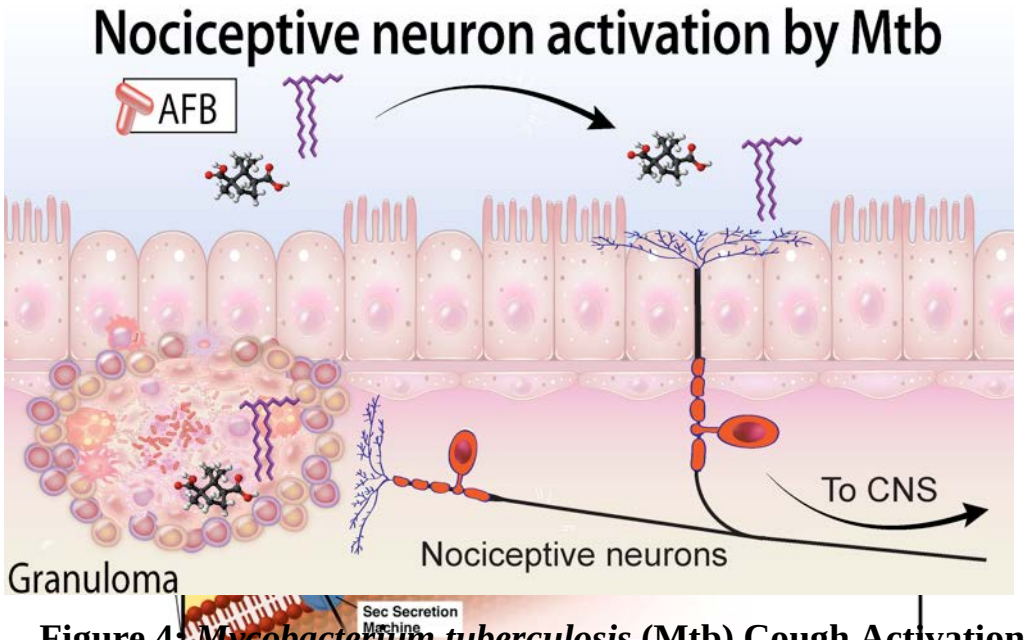

Figure 4: *Mycobacterium tuberculosis* (Mtb) Cough Activation

Figure 3: Many bioactive lipids and small molecules of the *Mycobacterium tuberculosis* cell wall

alone are sufficient to trigger neurons to fire and experimental animals to cough. Ongoing work in our laboratory is defining the mechanisms of biosynthesis of these molecules, their neuronal receptors and the impact of cough on *Mycobacterium tuberculosis* transmission in the context of experimental infection.

Conclusions

Infectious diseases are transmitted via various mechanisms, including the airborne route. Taking advantage of the principles of aerobiology, *Mycobacterium tuberculosis* has caused significant morbidity and mortality for centuries. Despite the importance of tuberculosis to global health, our understanding of why some individuals develop active versus latent disease, our ability to prevent direct transmission from an infected individual to an uninfected individual, and our treatment options (especially in the era of increasingly multidrug-resistant strains of tuberculosis) are incomplete. Moreover, while cough is a hallmark symptom of active pulmonary tuberculosis and a clear mechanism of disease spread, there is a paucity of research exploring the mechanisms by which *Mycobacterium tuberculosis* is able to trigger the airway nervous system and cough reflex. Better understanding of how *Mycobacterium tuberculosis* induces cough to mediate its transmission at the molecular level, a poorly understood yet fundamental aspect of *Mycobacterium tuberculosis* pathogenesis, is clearly needed. Once this mechanism is delineated, there are many far-reaching implications for disease control including development of novel therapeutics and infection prevention measures. Moreover, further studies may help elucidate similar mechanisms of cough induction for other infectious organisms.

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