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Nonhuman Primate Models of Respiratory Disease: Past, Present, and Future

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Abstract

The respiratory system consists of an integrated network of organs and structures that primarily function for gas exchange. In mammals, oxygen and carbon dioxide are transmitted through a complex respiratory tract, consisting of the nasal passages, pharynx, larynx, and lung. Exposure to ambient air throughout the lifespan imposes vulnerability of the respiratory system to environmental challenges that can contribute toward development of disease. The importance of the respiratory system to human health is supported by statistics from the Centers for Disease Control and Prevention; in 2015, chronic lower respiratory diseases were the third leading cause of death in the United States. In light of the significant mortality associated with respiratory conditions that afflict all ages of the human population, this review will focus on basic and preclinical research conducted in nonhuman primate models of respiratory disease. In comparison with other laboratory animals, the nonhuman primate lung most closely resembles the human lung in structure, physiology, and mucosal immune mechanisms. Studies defining the influence of inhaled microbes, pollutants, or allergens on the nonhuman primate lung have provided insight on disease pathogenesis, with the potential for elucidation of molecular targets leading to new treatment modalities. Vaccine trials in nonhuman primates have been crucial for confirmation of safety and protective efficacy against infectious diseases of the lung in a laboratory animal model that recapitulates pathology observed in humans. In looking to the future, nonhuman primate models of respiratory diseases will continue to be instrumental for translating biomedical research for improvement of human health.

Key words: asthma; COPD; infectious disease; inhalation; lung; nonhuman primate; toxicology

Introduction

The mammalian respiratory system consists of a complex series of passageways (nasal cavity, larynx, trachea, bronchi) for transportation of air to and from regions of gas exchange (alveoli). Beyond serving as a conduit for respiration, various regions of the lung mucosa are equipped with structural and

hematopoietic cell populations that have a protective function for overall immunity within the individual. Products of the environment, whether infectious or toxicological in nature, often elicit a protective response of the respiratory system that results in short-term morbidity or chronic disease. To understand the biological consequences of inhaled environmental challenges as well as effectively design strategies to mitigate pulmonary disease that contributes to morbidity and mortality in humans, it is critical to conduct basic and translational research in a laboratory animal model that closely recapitulates the respiratory tract of humans. Although genetically modified rodents continue to be the backbone of respiratory diseases research, nonhuman primate species are essential for understanding the progressive pathogenesis of conditions that are initiated during early life. Moreover, immune and physiologic parameters in the nonhuman primate closely reflect the complexity of human systems. While the costs for nonhuman primate research may be prohibitive of large-scale animal experiments, the potential for translation to human clinical trials is of high value. The following sections will discuss the comparative anatomy and physiology of the nonhuman primate lung relative to what is known in humans, followed by an overview of major contributions of this laboratory animal model in our understanding of inhalation toxicology, chronic airways disease, and infectious disease. It must be acknowledged that the comparative anatomy of the nonhuman primate lung has been comprehensively summarized in prior publications (Plopper and Harkema 2005; Plopper and Hyde 2008). An important goal for this current review is to build on the comparative anatomy knowledge base with translational studies that expand our understanding of airways physiology and respiratory immunity in a primate species.

Comparative Anatomy and Physiology of the Nonhuman Primate Lung

Airway Gross and Microscopic Anatomy

For humans as well as all small and large laboratory mammal species, the respiratory tract divides at the bifurcation of the trachea into a right and left lung (reviewed in Plopper and Harkema 2005). In both humans and rhesus macaque monkeys (*Macaca mulatta*), the left lung is divided into two distinct lobes. In the rhesus monkey, the right lung is divided into four lobes, whereas the human right lung is divided into three lobes. In comparison, the rodent left lung is not distinguished by individual lobes for the left lung, but does have four distinct lobes for the right lung, similar to that of the nonhuman primate. The overall lung mass in the rhesus monkey about 8% of body weight, in comparison with approximately 6% of body weight in humans and 30% of body weight in mice. A distinguishing feature of conducting airway anatomy for primate species is dichotomous, symmetric branching of the upper intralobar airways, which transitions to the alveolar regions via several generations of thin-walled, noncartilaginous bronchioles that contain outpocketings of alveoli (respiratory bronchioles). Respiratory bronchioles are not observed in rodent species and the branching pattern is monopodial, which can result in significantly different deposition profiles for inhaled materials. One feature common to both primate and rodent species is a high rate of particle deposition in anatomical regions transitioning from air conduction to gas exchange, whether respiratory bronchioles for primates or centriacinar regions for rodents. However, the cell populations located at that transition from conducting

airway to gas exchange consist of a mixture of ciliated, club, and alveolar epithelial cells in primates vs a distinct separation of bronchiolar cells from alveolar epithelial cells in the rodent. It is unknown if such differences in epithelial cell complexity contribute to species distinct biological responses to environmental challenges, but manifestation of small airways diseases such as respiratory bronchiolitis in response to infection and bronchiolitis obliterans following transplantation is not uncommon in primate species (reviewed in Burgel et al. 2013).

On a cellular level, the conducting airway tree of the lung displays the most species-dependent differences when comparing a range of laboratory animal models. From the trachea through midlevel intralobar airways, the overlying epithelia are a mixture of ciliated, mucous, and basal cells, along with extensive submucosal glands. The mucosa of conducting airways in primate species is substantial from the most proximal (trachea) to distal intralobar regions, containing both cartilage and extensive smooth muscle. In comparison, the epithelial lining of the rodent conducting airway tree is predominantly populated with club cells and ciliated cells and a minimal amount of lamina propria containing a small number of smooth muscle fibers. The individual cellular constituents that contribute to the structure of the lung play an important role in many different acute and chronic respiratory diseases, emphasizing the need to conduct both basic and translational studies in nonhuman primates to define mechanisms that promote pathology in humans. For example, comparison of the airway transcriptome from the rhesus macaque model and mouse model of house dust mite-induced asthma shows significantly greater overlap of gene profiles from monkeys with lung biopsies obtained from human asthma patients (Abbas et al. 2011).

Airways Physiology

Identifying the mechanical factors within the pulmonary system that contribute to ventilatory dysfunction is critical for understanding and ultimately treating the underlying cause of chronic airways disease. Moreover, mechanical changes of the lung in response to perturbations are indicative and diagnostic for disease status. The size of nonhuman primates commonly used in biomedical research permits characterization of pulmonary disease processes utilizing techniques common to the study of human pulmonary disease. Pulmonary function testing has been utilized in nonhuman primate research over the course of several decades. Initially, studies that measured lung volumes, capacities, and pulmonary mechanical variables in nonhuman primates were relatively descriptive in nature but important for providing a basis for expected values. The rhesus monkey chest wall is relatively stiff in comparison to small domestic animals but similar to the human chest wall. This feature of primate anatomy physiologically gives rise to a relaxed lung volume known as functional residual capacity, which comprises a large percentage of the total lung capacity (Koike et al. 1998; Pare et al. 1978). Dynamic flow parameters, similar to forced expiratory volume commonly used in human patients, have also been measured in nonhuman primates and found to be similar to dogs but larger than that in humans when normalized to lung size (Kosch et al. 1979).

Common methods of mechanical characterization rely on a simple model of the pulmonary system giving a pulmonary resistance and dynamic compliance. A more complex method that holds promise for extensively characterizing pulmonary mechanical function with minimal invasiveness is forced

oscillometry, which is gaining popularity in human patients, especially those that cannot perform maneuvers comprising common pulmonary function tests. Forced oscillometry is a technique that has been under development for many years, and monkeys have been included as a part of the evaluation process (DuBois et al. 1956; Wegner et al. 1984). Using a model system that allows separation of compliance, airway resistance, and tissue resistance, cynomolgus macaque monkeys (*Macaca fasicularis*) were found to respond to bronchoactive agents primarily with changes in airway resistance indicating a large airway contribution and the frequency dependence of the response suggesting heterogeneous constriction (Black et al. 2001; Madwed and Jackson 1997). Other studies in monkeys suggest that peripheral airways contribute about one-third of lung resistance in response to bronchoactive agents (Pare et al. 1976). Given the availability of techniques enabling identification of peripheral airway dysfunction and heterogeneity of ventilation in addition to more standard parameters, and the fidelity of nonhuman primate lung development and anatomy to that of human, this laboratory animal model is particularly relevant to the study of pulmonary disease processes in humans, especially where small airways play a significant role (Shaw et al. 2002).

Role of Nonhuman Primates in Inhalation Toxicology

Ozone

Ozone is a major constituent of photochemical air pollution. Acute and chronic inhalation studies in nonhuman primates have played a critical role in defining ozone-induced injury and inflammatory and repair responses. More recent nonhuman primate studies have focused on the effects of early-life ozone exposure on lung development.

Numerous studies have demonstrated ozone-induced lesions or inflammation in the conducting airways of rhesus monkeys and bonnet macaque monkeys (*Macaca radiata*) exposed to ozone concentrations of 0.15 to 3.0 ppm for periods of 2 hours to 7 days (Castleman et al. 1973, 1975, 1977, 1980; Dungworth et al. 1975; Mellick et al. 1977). A gradient of damage, with the large bronchi having the greatest damage and the distal bronchioles having the least, has been observed in nonhuman primates exposed to high ambient concentrations of ozone (0.40 and 0.64 ppm) for 8 hours/day for 7 days. Castleman et al. exposed bonnet monkeys to 0.16 and 0.30 ppm ozone 8 hours/day for 7 days and observed altered cilia and an increase in nonciliated cells (Castleman et al. 1980). Again, the most severe damage, involving the largest number of ciliated cells, was located in the trachea and proximal bronchi, while less extensive changes were present in more distal bronchi and respiratory bronchioles. A consistent observation in subacute exposure studies using nonhuman primates is that surface alterations viewed by scanning electron microscopy consist of randomly scattered patches and longitudinal tracts.

As the number of days of ozone exposure extends beyond 7 days, the lesions in trachea and proximal bronchi recover, with the epithelium of the large conducting airways becoming relatively insensitive to continued exposure (Wilson et al. 1984). In contrast, monkeys exposed to 0.15 ppm ozone for 6 or 90 days, 8 hours per day, had significant nasal epithelial lesions of ciliated cell necrosis, attenuated cilia, secretory cell hyperplasia and bronchiolitis consisting of hyperplasia, hypertrophy of nonciliated bronchiolar epithelial cells, thickening of the

interstitium, and intraluminal accumulations of macrophages (Harkema et al. 1987, 1993). The development of chronic bronchiolitis with prolonged (1–18 months) exposure to high ambient concentrations of ozone has been demonstrated in multiple nonhuman primate studies (Eustis et al. 1981; Fujinaka et al. 1985; Harkema et al. 1993; Tyler et al. 1988). Tyler et al. exposed young nonhuman primates to 0.25 ppm continuously and episodically every other month for 18 months and observed that despite delivering one-half of the ozone dose, the episodic exposure produced larger biochemical and physiological alterations and equivalent morphometric changes (Tyler et al. 1988).

To examine the effect of early-life ozone exposure on lung development, 1-month old infant rhesus macaques were exposed to 11 episodes of 5 days (8 hours/day) at 0.50 ppm followed by 9 days of filtered air (Schelegle et al. 2003). Compared with control infants, ozone-exposed animals had reduced epithelial innervation, four fewer nonalveolarized airway generations, hyperplastic bronchiolar epithelium, altered smooth muscle bundle orientation in terminal and respiratory bronchioles, and increased alveolar numbers (Avdalovic et al. 2012; Fanucchi et al. 2006; Larson et al. 2004). These changes were associated with altered serotonin, substance P, and fibroblast growth factor signaling in conducting airways (Evans et al. 2003; Moore et al. 2012; Murphy et al. 2012, 2013). The cessation of exposure for 6 months resulted the normalization of fibroblast growth factor signaling and epithelial hyperinnervation, while airway serotonin signaling remained altered for as long as 2.5 following the end of exposure (Evans et al. 2004; Kajekar et al. 2007; Moore et al. 2014). In total, these studies of early-life episodic ozone exposure provide evidence for prolonged structural and functional airway changes that could increase the risk for chronic lung disease as the animal ages.

Tobacco Smoke

Exposure to tobacco smoke is a major contributor to cardiopulmonary disease. Irritation and chronic inflammation of the airways and alveoli lead to a myriad of conditions including bronchitis, chronic obstructive pulmonary disease (COPD), and emphysema, as well as multiple forms of benign and malignant lung cancer. According to the World Health Organization, COPD is the fourth leading cause of death in the world today. Exposure to tobacco smoke can be either active or passive and represents the more preventable type of lung disease known through smoking cessation and regulation.

Nonhuman primates have been key in advancing our understanding of the mechanisms of smoke-induced lung disease due to early-life exposure to second-hand smoke, also known as environmental tobacco smoke. Early-life exposure to second-hand smoke can have significant consequences on the normal development of a wide variety of organ systems but in particular the respiratory system. Exposure to low levels of environmental tobacco smoke (1 mg/m^3) during pregnancy (beginning at gestation day 50) through the first 2 months of postnatal life downregulates NF-kappaB-dependent antiapoptotic genes to induce the activation of caspase cleavage of cellular death substrates (poly(ADP)-ribose polymerase and caspase-activated DNase), thus increasing the rate of apoptosis in the neonatal lung parenchyma. These events directly compromise normal cell proliferation with the potential to impair normal lung growth and function in early life (Zhong et al. 2006).

Exposure to environmental tobacco smoke during early life in the nonhuman primate also enhances local T(H)2 immunity

by impairing normal pulmonary T(H)1 immune maturation. This effect is greater in animals beginning environmental tobacco smoke (ETS) exposure in utero in contrast to infants exposed only postnatally. Neonatal monkeys exposed in utero and during the first 6 months of postnatal life demonstrate significant downregulation in mRNA for IFN-gamma, IL-2, IFN-gamma-inducible protein 10, monokine induced by IFN-gamma, IFN-gamma-inducible T-cell chemoattractant, CXC chemokine receptor 3, IL-12 bioactive p70 subunit, and T-bet. In contrast, infant monkeys exposed only during postnatal life (6–13 months of age) to ETS show a significant downregulation only in IFN-gamma, CXC chemokine receptor 3, and IL-12p70, while levels of mRNA for thymus and activation-regulated chemokine are increased and IL-10 protein is reduced (Wang et al. 2008).

Perinatal ETS exposure in monkeys can induce both systemic and local responses, with significant elevations in plasma levels of C5a and brain-derived neurotrophic factor, as well as significant increases in the expression of proinflammatory cytokine TNF-alpha and T(H)2 cytokine IL-5, chemokine monocyte chemoattractant protein 1 in the lungs, along with a significant increase in the presence of substance P-positive nerves along the bronchial airways to innervate the epithelial lining of the lungs. Perinatal ETS exposure also significantly increases the number of mast cells, eosinophils, monocytes, and lymphocytes in the lungs of infant monkeys (Yu et al. 2008).

Perinatal exposure to ETS can also induce acute systemic inflammatory responses in the nonhuman primate to significantly alter a variety of immune effectors. Fetal and postnatal exposure to ETS has been found to alter the normal maturation of mRNA levels of IFN-gamma, IL-2, and IL-10, as well as the ratio of CD4 to CD8 lymphocytes, compared with filtered-air control levels. Blood lymphocyte subset distribution have also been found to be significantly different, based on the timing of the onset of exposure to ETS in early life. Even short-term exposure to ETS (2 weeks) in postnatal infant monkeys has been found to significantly increase the levels of peripheral blood neutrophils and IL-6 mRNA. These findings suggest normal immune system development can be significantly compromised by in utero and postnatal exposure to ETS to contribute to ETS-related childhood diseases (Wang et al. 2007).

Other Inhaled Toxicants

Ozone and tobacco smoke are the most well-studied environmental exposures in nonhuman primate models, likely due to the strong association of these common air pollutants with chronic respiratory diseases in humans such as asthma and COPD. There are limited data available on other inhalation toxicology studies in nonhuman primates. For occupational exposures, chlorine gas and formaldehyde gas have been evaluated in chronic models in the rhesus macaque, eliciting significantly different pathology compared with rat models (Klonne et al. 1987; Monticello et al. 1989). The carcinogenicity of long-term diesel and coal dust exposure was examined in the cynomolgus monkey; in comparison with the rat, the monkeys showed minimal evidence of cellular abnormalities (Lewis et al. 1986). Similar findings were observed in an 11-year follow-up study in cynomolgus monkeys exposed to asbestos (Stettler et al. 2008). Despite the pervasiveness of particulate matter (PM2.5 and PM10) in the environment, there are no known reports of experimental exposures for this airborne product of combustion in nonhuman primate models. Recently, a cohort of outdoor-housed adolescent rhesus macaque monkeys exposed to ambient wildfire smoke (a high source or PM2.5 and PM10)

were evaluated for lung function and immune profiles; animals exposed at infancy displayed reduced lung volumes and dysregulation of innate immunity, suggesting long-term health impacts from inhaled particulate matter during early life (Black et al. 2017).

Future Studies

Electronic cigarette use tripled among middle and high school-age children from 2013 to 2014 (Arrazola et al. 2015). Adolescents perceive electronic cigarettes to be safer than traditional cigarettes, resulting in use that is expanded to individuals who may not use traditional cigarettes (Barrington-Trimis et al. 2015). Cigarette smoking is overrepresented in adolescents with psychiatric disorders, but e-cigarette use extends to an emotionally healthier subset expanding use beyond those that would use traditional cigarettes (Leventhal et al. 2016; Upadhyaya et al. 2002). Epidemiological estimates indicate electronic cigarette vapor (ECV) carries a risk for asthma in young people, whereas it may be too early to estimate risk for COPD. ECV use for 5 minutes in healthy smokers elicits pulmonary function deficits similar to smoking (Cho and Paik 2016; Vardavas et al. 2012). While perceived as safe alternatives to traditional cigarettes, current evidence indicates e-cigarettes will contribute to lung disease. A nonhuman primate model would be well-positioned to directly assess the acute and chronic effects of inhalation exposures in the adolescent population. Complex behavioral patterns exhibited by monkeys can also be associated with pulmonary dysfunction and may couple with exposures such as ECV to exacerbate pulmonary disease processes, pointing to a monkey model for optimal discovery of these processes.

Nonhuman Primate Models of Airways Inflammation

Asthma

With the advent of pulmonary function testing techniques in nonhuman primates, investigators began characterizing cynomolgus monkeys that were naturally infected with the nematode *Ascaris suum* and showed allergic reactions to the *A. suum* antigen (Patterson and Talbot 1969). The model proved to be a good representation of human atopic asthma and became widely used by the pharmaceutical industry, which helped characterize the allergic airway disease (Gundel et al. 1992; Krell et al. 1986; Osborn et al. 1992; Turner et al. 1996; Young et al. 1999). The acute allergic airway response in monkeys was found to be primarily mediated by histamine and cysteinyl leukotrienes (Osborn et al. 1992). The relative potency of cysteinyl leukotrienes (cys-LT) compared to histamine was variable in some studies where there may be some regional and species-specific effects, but inhalation of cys-LT by monkeys does mimic an asthmatic phenotype (Johnson et al. 1988; Krell et al. 1986; Seehase et al. 2011). The reaction to cys-LT in monkeys appears to be mediated through a cholinergic reflex in the airways, whereas the parenchymal response showed less response to cholinergic inhibition (atropine) (Johnson et al. 1988). The monkey model also revealed responders displaying a late-phase response similar to asthmatics. The late-phase airway obstruction was tied to neutrophil influx that is primed by the presence of eosinophils in the airway and inhibited by blocking endothelial leukocyte adhesion molecule 1 (Gundel et al. 1991, 1992).

The "natural" model of *A. suum* asthma has given way to models utilizing active sensitization with a number of different allergens, such as the common human allergen, house dust mite. In 2001, the first nonhuman primate model of experimentally induced asthma using house dust mite (*Dermatophagoides farine*) in rhesus monkeys was reported, which was subsequently replicated by other investigators using the cynomolgus monkey (Schelegle et al. 2001; Van Scott et al. 2004). In the rhesus monkey, a period of chronic aerosol challenge with house dust mite resulted in multiple parameters of clinical asthma in humans, including eosinophilic inflammation in the lung, remodeling of conducting airways, and enhanced physiologic reactivity to a nonspecific stimuli (methacholine) (Schelegle et al. 2001). This rhesus model of house dust mite-induced atopic asthma has been utilized in preclinical trials for asthma therapeutics (Fanucchi et al. 2004; Seshasayee et al. 2007). An infant rhesus monkey model of childhood asthma that combines the adjuvant properties of ozone with house dust mite exposure has also been reported; inhaled corticosteroid treatment of infant monkeys that display an asthma phenotype resulted in attenuation of some symptoms but also altered normal lung growth (Plopper et al. 2012; Schelegle et al. 2003).

COPD

COPD is associated with cigarette smoking, and a few studies have attempted to model COPD through exposure of monkeys to tobacco smoke. An early study trained baboons to smoke and assessed them after 6 pack years of smoking (a common metric in COPD studies indicating the product of the average packs per day smoked and years smoking). Interestingly, the bronchial reactivity was blunted, and residual lung volume was preserved upon exposure to the cholinergic agonist methacholine in the smoking monkeys (Roehrs et al. 1981). Inhalation of nicotine was found to mediate the blunted methacholine response while not affecting baseline lung function, whereas the response to histamine was unaffected (Wallis et al. 1982). A more recent examination of heavy smoking, approximating 4 packs/day, in cynomolgus monkeys over the course of 4 and 12 weeks provided some insight into the pathological processes in the monkey model. After 12 weeks, pulmonary function changes showed minimal differences from sham exposure (a trend toward reduced FEV), while pathological changes were more pronounced. Inflammation, mucus metaplasia, glandular hypertrophy/hyperplasia, and peribronchial fibrosis was evident in addition to a "pre-emphysemetous" profile indicated by elevated matrix metalloprotein-9, apoptosis of alveolar septal cells, and elevated oxidative stress in lung tissue (Polverino et al. 2015). Using this approach, it was recently reported that a combination of cigarette smoke and influenza infection increases connective tissue growth factor, which could accelerate epithelial cell senescence (Jang et al. 2017).

Future Studies

In line with a continuing interest in personalized medicine by the biomedical research community, there has been a major push to phenotype human asthma and COPD subjects using molecular markers based on multiple parameters of clinical severity. One category of the human patient population that has proven to be experimentally challenging to replicate in animal models is the nonatopic or "intrinsic" asthmatic. To date, there is very little known regarding the underlying mechanisms that elicit nonatopic asthma, beyond elevated body mass index,

and increased serum IL-6 (Fahy 2015; Peters et al. 2016). In a retrospective study, Capitanio and colleagues reported that a behaviorally inhibited temperament in rhesus monkeys may be predictive of intrinsic airways hyperresponsiveness to nonspecific challenge; this finding was subsequently replicated in a prospective study (Capitanio et al. 2011; Chun et al. 2013). The underlying pulmonary function deficit associated with the airway hyperresponsiveness has not been determined in this natural model of nonatopic asthma (Royer et al. 2016), but studies to investigate the immune and genetic basis of this phenotype should be conducted in the future.

Nonhuman Primate Models of Respiratory Infection

Nonhuman primates have long served as laboratory animal models for infectious diseases in humans where the lung is the primary site of infection and manifestation of illness. In comparison with other laboratory animals such as rodent or canine species, the immune system of the nonhuman primate shows significant homology with that of the human, which is a critical characteristic for understanding host-pathogen responses that lead to identification of therapeutic targets (Messaoudi et al. 2011). Because of similarities in immune mechanisms, nonhuman primate models of respiratory infection provide a critical biomedical resource for accelerating preliminary safety and efficacy testing of vaccines as well as other pharmaceutical interventions that cannot be ethically evaluated in human subjects prior to clinical trials. On a global scale, pathogens that target the respiratory system are of particular concern to human health due to the possibility of airborne transmission, leading to rapid dissemination of infection via international travel. The complexity of mechanisms used by certain pathogens of the respiratory tract to evade the immune system of the host has contributed to slow but incremental success in identifying cellular pathways that may be targeted for drug and vaccine development. More recent experimental studies in nonhuman primate models have focused on respiratory viruses that have evolved from domesticated or wild animal hosts, along with continued efforts to prevent the establishment of respiratory infectious disease in the human population using novel vaccination methods. Fungal and bacterial respiratory infections have also been investigated in nonhuman primate models, primarily in the context of secondary opportunistic pathogens in the immunocompromised host. Here, we will highlight nonhuman primate infection models of the most common viruses and microbes that target the respiratory tract, with an emphasis on clinical treatment and prevention strategies that may be translated into the human population.

Tuberculosis (*Mycobacterium tuberculosis*)

It is estimated by the World Health Organization that more than 1 million people die each year from tuberculosis (TB), with more than 8 million individuals becoming newly infected each year (reviewed in Zumla et al. 2013). Despite the known existence of this disease since the 1700s, it remains as one of the top 10 causes of death worldwide, making the search for an effective vaccine and drugs that do not elicit resistance of the mycobacterium a priority. One of the oldest experimental nonhuman primate models of human respiratory pathogen infection was developed to address the imperfections of murine models of *Mycobacterium tuberculosis*, the causative agent for TB in humans. A major first step in the establishment of

nonhuman primate models of TB was reported by Walsh et al., in which the cynomolgus monkey was used to demonstrate the feasibility of infection by intratracheal administration of either high or low doses of *M. tuberculosis*; while high doses of the *M. tuberculosis* elicited fulminant conditions, low doses resulted in chronic symptoms that were similar to what has been described for human patients (Walsh et al. 1996). The cynomolgus model was subsequently refined by Capuano et al. using bronchoscopy for low-dose instillation *M. tuberculosis* followed by long-term immune and clinical monitoring of animals, resulting in a cohort that displayed the spectrum of pathological conditions observed in human populations (Capuano et al. 2003). In the early 1920s, the bacillus Calmette-Guerin (BCG) vaccine to prevent TB was developed using a weakened strain of the bovine *Mycobacterium bovis*; while currently still in use in many countries, the efficacy of BCG to prevent the development of TB is highly variable (Colditz et al. 1994). Findings from human epidemiology have been borne out by experimental nonhuman primate studies in a rhesus macaque monkey model of experimental TB, which clearly supported the observation of limited efficacy for BCG vaccine (Langermans et al. 2001). Numerous attempts using innovative vaccination approaches have failed; however, a recent mucosal aerosolization study of a *M. tuberculosis* strain engineered with a mutant SigH region effectively protected rhesus macaques from lethal TB challenge, suggesting that human clinical trials are imminent (Kaushal et al. 2015). In addition to serving as a critical laboratory animal model for human TB, the nonhuman primate has been critical for the development of blood diagnostics for detection of interferon production in response to *M. tuberculosis*, a step that has virtually eliminated the need for the less sensitive and specific tuberculin skin test (Garcia et al. 2004).

Measles (Measles Virus)

Measles is a disease resulting from infection with the measles virus, which is classified as an RNA virus within the genus Morbillivirus and Paramyxoviridae family. The measles virus is highly contagious and readily spread by airborne transmission following infection within the respiratory tract. Infection with the measles virus can result in significant morbidity and immunosuppression, leading to enhanced susceptibility to mortality from childhood infectious disease (Mina et al. 2015). Despite the availability of effective vaccines since the early 1960s, resource-poor countries and the recent spate of "antivaccine" proponents has contributed to a dramatic increase in outbreaks both globally and within the United States, necessitating investigation of more robust vaccine strategies to enhance gaps in immunity (Durrheim 2016; Gastanaduy et al. 2016; Mulholland et al. 2012). The nonhuman primate is highly susceptible to infection with the measles virus, and experimental inoculation can result in acute clinical signs that are comparable to that observed in humans (Kobune et al. 1996). Surprisingly, little is known about the cellular and molecular mechanisms by which the measles virus elicits pathology. Because the respiratory tract has long been known as the primary source of viral particles, it had been assumed that epithelial cells were the primary target, despite the lack of expression for CD150, the known high affinity receptor for viral entry (Erlenhoefer et al. 2001). In an elegant study in cynomolgus macaques using a recombinant green fluorescent protein, labelled measles virus clearly demonstrated that alveolar macrophages and dendritic cells were the initial sites of viral replication, followed by spread to lymphoid aggregates in the oral cavity (Lemon et al. 2011; Ludlow

et al. 2013). More recently, the preferential targeting of antigen-presenting cells by the measles virus was further supported by i.m. inoculation of live-attenuated virus in cynomolgus macaques (Rennick et al. 2015). Innovative approaches to achieve high coverage of vaccination has also been developed in the nonhuman primate model of measles, including the use of a microneedle patch that does not require injections (Edens et al. 2015).

Influenza (Influenza Virus)

Influenza is a respiratory disease caused by infection with either Influenza A or B virus strains, which are RNA viruses of the orthomyxovirus family. In contrast with seasonal influenza, infection with pandemic influenza A virus strains is a significant concern for human health due to limited immunity on a global scale, resulting in complications that require hospitalization and mortality in at-risk populations. Experimental animal models of viral infection using the nonhuman primate have played an essential role in understanding the pathogenesis of the pandemic influenza A strain H1N1 as well as other highly pathogenic strains such as avian H5N1 (Davis et al. 2015). From a mechanistic perspective, the nonhuman primate has been particularly important for defining immunomodulatory pathways that dictate health outcomes in vulnerable populations with weakened immunity toward vaccine preparations, such as very young children and geriatric individuals (Asquith et al. 2012; Clay et al. 2014b; Holbrook et al. 2015). Not surprisingly, both infant rhesus macaques and African green monkeys are more susceptible to the H1N1 virus, revealing delayed type 1 interferon synthesis and impaired mucosal immunoglobulin responses following infection. Seasonal influenza vaccines are not recommended for children under the age of 6 months, which is a target population that is most vulnerable to hospitalization and mortality due to infection. As such, a major emphasis for nonhuman primate models of influenza infection is the investigation of vaccine adjuvants to enhance immunogenicity in vulnerable populations. To date, inclusion of adjuvants such as flagellin appears to have modest efficacy in pediatric H1N1 infection models, resulting in enhanced parameters of humeral immunity (Holbrook et al. 2016a, 2016b). However, cationic lipid/DNA complexes have been shown to dramatically reduce viral replication following vaccination with a trivalent inactivated influenza vaccine in geriatric animals challenged with H1N1 (Carroll et al. 2014).

Viral Bronchiolitis (Respiratory Syncytial Virus)

Infection with respiratory syncytial virus, an RNA virus of the paramyxoviridate family, is one of the most common respiratory infections during early life, with an estimated 20% of children infected (reviewed in Meissner 2016). All populations, from infancy to geriatric, are vulnerable to infection with respiratory syncytial virus, but infants are particularly susceptible to the development of viral bronchiolitis, often requiring hospitalization. Moreover, childhood infection with respiratory syncytial virus is frequently a predictor for development of asthma later in life (Jackson et al. 2016). Several nonhuman primate species have been utilized as models of respiratory syncytial virus infection, including the rhesus macaque, Bonnet monkey (*Macaca radiata*), and baboon (De Swart et al. 2002; McArthur-Vaughan and Gershwin 2002; Papin et al. 2013; Simoes et al. 1999). Vaccine trials for respiratory syncytial virus have proceeded with caution following several deaths resulting from an

early trial using a formalin inactivated virus; subsequent testing in nonhuman primates showed that viral challenge elicited Th2-derived hypersensitivity reactions (De Swart et al. 2002). To date, there is no safe and effective vaccine for respiratory syncytial virus; high-risk infants may receive prophylaxis in the form of a humanized monoclonal antibody targeted against the viral surface protein.

SARS/MERS (SARS CoV/MERS CoV)

Both severe acute respiratory syndrome (SARS) and Middle East Respiratory syndrome (MERS) are pulmonary diseases caused by infection with members of the Coronoviridae family of single-stranded RNA viruses. Outbreaks of either SARS or MERS are relatively isolated but result in severe disease and mortality in susceptible populations. In the first reported outbreak of SARS in 2003, confirmation of SARS coronavirus as the etiologic agent for disease was determined by infection of rhesus macaque monkeys, resulting in symptoms that were comparable to human infection(Rowe et al. 2004). Other studies in African green monkeys have established that immune deficiencies associated with the aging process result in enhanced morbidity following infection with the SARS coronavirus (Clay et al. 2014a). The first known report of MERS took place in 2012, with additional cases emerging within the same Middle Eastern region (Arabi et al. 2017). The MERS coronavirus can infect both the rhesus macaque and common marmoset (*Callithrix jacchus*), resulting in clinical symptoms; the marmoset has been reported to develop lethal pneumonia following infection (Falzarano et al. 2014; Yao et al. 2014).

Fungal and Bacterial Pneumonia (*Pneumocystis jirovecii, Streptococcus pneumoniae, Klebsiella pneumoniae*)

The fungus *Pneumocystis jirovecii* is abundant within the environment but can be highly pathogenic in immunocompromised humans, resulting in life-threatening pneumonia. Pneumocystis pneumonia is frequently associated with human immunodeficiency virus infection; however, individuals undergoing chemotherapy and susceptible populations (infant, geriatric) are also vulnerable to infection (reviewed in Eddens and Kolls 2015). Confirmation of the role of immune compromise in the pathogenesis of Pneumocystis pneumonia was experimentally determined in the macaque model of human immunodeficiency virus infection using the simian immunodeficiency virus (SIV) (Board et al. 2003). It was subsequently shown that humeral immunity to the *P. jirovecii* protease kexin (KEX1) protein is strongly linked with health outcomes in the SIV-infected monkey (with corollaries in human patients) (Kling et al. 2010). Vaccination with recombinant KEX1 peptide resulted in protective humeral immunity against *P. jirovecii* infection, even in immunosuppressed SIV-infected monkeys (Kling and Norris 2016).

Similar to fungal pneumonia, susceptible populations are also more at risk for development of bacterial pneumonia; it is estimated that *Streptococcus pneumoniae* contributes to approximately 11% of deaths in children worldwide (O'Brien et al. 2009). In the late 1970s, models of *S. pneumoniae* and *Klebsiella pneumoniae* were initially described in squirrel monkeys (*Saimiri sciureus*), which, in contrast with rodent models, elicited a lobar pneumonia comparable to that of humans (Berendt et al. 1978, 1975). A rhesus macaque model of *S. pneumoniae* infection was subsequently developed and successfully used for evaluation of a novel protein-based pneumococcal vaccine (Denoel et al. 2011; Philipp et al. 2006). More recently, a severe pneumonia

model has been described in the adult baboon (*Papio cynocephalus*), primarily for the purpose of identifying new biomarkers of early stage infection, with the goal of rapid diagnosis and treatment (Kraft et al. 2014; Reyes et al. 2016).

Whooping Cough (*Bordatella pertussis*)

Whooping cough is an acute respiratory illness of childhood that is the result of infection with *Bordatella pertussis*, a highly contagious gram-negative bacterium that blocks normal mucociliary clearance in the lung by production of a cytotoxin. Vaccination for *B. pertussis* has been available since the 1940s, and an acellular form in conjunction with diphtheria and tetanus has been incorporated as a part of routine childhood immunization schedules for decades (reviewed in Kilgore et al. 2016). The incidence of whooping cough has steadily increased over the years, with epidemiologic reports implicating waning immunity in adolescence contributing to outbreaks in the United States (Klein et al. 2012). To better understand the immune basis for declining immunity in juveniles, multiple species of nonhuman primates have been evaluated as animal models of *B. pertussis*. The Taiwanese macaque (*Macaca cyclopis*) may become infected with *B. pertussis* and develop clinical symptoms; however, availability of this species for research is limited as it is now considered endangered (Huang et al. 1962). In contrast with the Taiwanese macaque, approximately 25% of rhesus macaque monkeys infected with *B. pertussis* display clinical symptoms that are consistent with human disease (Warfel et al. 2012). Baboons appear to be a much more reliable nonhuman primate infection model for *B. pertussis* and have recently yielded important immunological insights on the mechanisms of diminished vaccine potency in the human population. Infection of baboons with *B. pertussis* or vaccination with whole pertussis results in long-lasting Th17 and Th1 immunity; however, the currently used acellular pertussis vaccine elicited a mixed Th1/Th2 immunity, suggesting that T cell effector mechanisms may be critical for robust disease protection (Warfel and Merkel 2013; Warfel et al. 2014). More recent studies in the baboon model have evaluated the prophylactic efficacy of humanized pertussis-neutralizing antibody cocktails as an adjuvant to traditional antibiotic treatment following *B. pertussis* infection (Nguyen et al. 2015).

Future Studies

Only within the past two decades, several respiratory viruses have emerged from animal reservoirs to infect an unprepared human population. With the density of the human population increasing worldwide and our almost unlimited capacity for global travel, outbreaks will continue to take place. The capacity for respiratory pathogens to precipitously impose catastrophic disease in the human population necessitates continued biomedical research in the investigation of novel chemical inhibitors of microbial growth, biologics to reduce life-threatening acute inflammation, and vaccines. Because the nonhuman primate lung and immune system so closely recapitulates that of humans, we will remain reliant on this essential laboratory animal model to assess for pathogenesis of infectious agents and efficacy of therapeutics in the future.

Conclusions

Despite significant progress in the development of robust nonhuman primate models of respiratory disease over the past

several decades, viable pharmaceutical candidates for permanent restoration of lung function and mucosal immunity remain elusive. The comparative aspects of nonhuman primate lung anatomy, physiology, and immunology reinforce the necessity for conducting pharmaceutical studies in this laboratory animal model, but the prolonged nature of chronic respiratory conditions presents a major obstacle for conducting experimental investigations in this species in a practical manner. To address these challenges, expanded use of new world monkey species with accelerated lifespans (e.g., marmosets) may ultimately be the key to success, with the potential for yielding critical information on the natural progression of obstructive airways and lung function decline in the human population.

Acknowledgments

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