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Moving towards a new generation of animal models for asthma and COPD with improved clinical relevance

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ABSTRACT

Asthma and chronic obstructive pulmonary disease (COPD) are complex inflammatory airway diseases characterised by airflow obstruction that remain leading causes of hospitalization and death worldwide. Animal modelling systems that accurately reflect disease pathophysiology continue to be essential to the development of new therapies for both conditions. In this review, we describe preclinical in vivo models that recapitulate many of the features of asthma and COPD. Specifically, we discuss the pro's and con's of the standard models and highlight recently developed systems designed to more accurately reflect the complexity of both diseases. For instance, clinically relevant allergens (i.e. house dust mite) are now being used to mimic the inflammatory changes and airway remodelling that result after chronic allergen exposures. Additionally, systems are being developed to mimic steroid-resistant and viral exacerbations of allergic inflammation – aspects of asthma where there is an acute need for new therapies. Similarly, COPD models have evolved to align with the improved clinical understanding of the factors contributing to disease progression. This includes using cigarette smoke to model not only airway inflammation and remodelling, but some systemic changes (e.g. hypertension and skeletal muscle alterations) that are thought to influence disease. Further, mouse genetics are being exploited to gain insights into the genetics of COPD susceptibility. The new models of asthma and COPD described herein demonstrate that improved clinical understanding of the diseases and better preclinical models is an iterative process that will hopefully lead to therapies that can effectively manage severe asthma and COPD.

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Contents

1. Introduction	0
2. Asthma	0
3. COPD	0
4. Next steps for modelling allergic asthma and COPD	0
5. Uncited references	0
Acknowledgments	0
References	0

1. Introduction

Chronic respiratory diseases are major causes of morbidity and mortality, comprising 7% of deaths and 4% of disability adjusted life years (DALYs) worldwide (WHO, 2008a,b). Asthma and chronic obstructive pulmonary disease (COPD) affect approximately 300 and

210 million people, respectively, with most of the deaths associated with both disorders occurring in low- and middle-income countries. In 2005 asthma was responsible for 255,000 deaths, whereas COPD was the cause of approximately 3 million deaths worldwide. The majority of asthma deaths occur in individuals with a severe form of the disease (5–10% of asthmatics), which is not effectively managed with the standard asthma therapy (i.e., glucocorticoid and β_2 -adrenoceptor agonist combination therapy). Similarly in COPD, there are no treatments that can halt the progression of the disease. Additionally, acute exacerbations of both asthma and COPD (where disease

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symptoms become acutely more severe) are major causes of hospitalizations and death in patients. Again, there is little that can be done to effectively limit the severity or reduce the frequency of these events. With asthma being the most common chronic disease amongst children and COPD predicted to become the 3rd leading cause of death by 2030, clearly these two conditions represent very large unmet medical needs that require urgent attention (WHO, 2008a,b).

In recent years, drug discovery efforts have largely centered around two different approaches – the development of “me too” drugs, such as improvements on existing therapies like bronchodilators and glucocorticoids, and novel therapeutic approaches aimed at specific molecular targets. While in vivo models of disease are still vital for the development of all therapies, they have been particularly important to the latter approaches. For instance, it has become commonplace to validate the “central role” of a particular molecular target in the pathogenesis of either asthma or COPD by characterizing the responses of genetically modified mice (with the target gene either knocked-out or overexpressed) in the most relevant in vivo models. Clearly, data from clinical specimens demonstrating the target has an association with the disease or correlation with disease progression is as, if not more, important for validating a particular target for drug discovery purposes; however, data from animal models provide the cause–effect relationship between target and disease-like phenotype that is a very powerful form of evidence that cannot be easily obtained in the clinic. Animal models have historically played an instrumental role in broadening our mechanistic understanding of both disorders. For instance, the observation that the instillation of an elastolytic proteinase, papain into the lungs of hamsters caused pronounced emphysema (Gross et al., 1965) was essential to the formation of the proteinase–antiproteinase imbalance hypothesis, which has dominated thinking about the pathogenesis of COPD in field for over 40 years. Similarly, the role of Th2 cytokines in the pathogenesis of asthma was also discovered as a result of studies performed in animal models of allergic lung inflammation.

While animal models for asthma and COPD have improved our understanding of the mechanisms driving disease, they are not without significant limitations. This is evidenced by the failure of some therapeutic approaches in clinical trials that were previously demonstrated to be efficacious in the animal models (Churg et al., 2004; Tanaka et al., 2004; Flood-Page et al., 2007; Rennard et al., 2007). Some suggest that these failures indicate that the models are not predictive and inadequate for the purposes of drug discovery (Wenzel & Holgate, 2006; Curtis et al., 2007). However, one must remember that the approach used to model diseases in small animals is to develop analogous systems whose aims are to induce disease-like changes that are reflective of the pathophysiological changes associated with asthma and COPD. Further, there is an incomplete understanding of the different clinical phenotypes of asthma and COPD and this reflects the paucity of animal models that have been developed to mimic these poorly defined patient sub-populations. Consequently, our ability to use these models as predictive systems is limited by our understanding of the mechanisms driving the pathogenesis of these respiratory syndromes.

Although our understanding of the pathogenesis of asthma and COPD has evolved considerably over the last 50 years, we still have very limited knowledge regarding the mechanisms driving these disorders. In fact, both conditions would be more accurately characterised as chronic respiratory syndromes, defined by specific physiological changes which are used to diagnose patients. The lack of an unequivocal mechanistic definition of both diseases is in part why these models are limited to mimicking certain pathophysiological features commonly associated with asthma and COPD in small animals through mechanisms that may not be entirely consistent with those involved in disease pathogenesis in man. However, past successes and failures have yielded several insights into the mechanisms underlying both disease pathogenesis and the disease-

like changes associated with the animal models. The challenge is to now use this knowledge to improve the in vivo models used to emulate key aspects of these chronic respiratory diseases.

Here, we review the recent progress made in the ability to model features of asthma and COPD in small laboratory animals – specifically, mice, rats and guinea pigs. Current efforts have focused on using complex agents that are known to cause the diseases in man to induce disease-like changes in small animals. The aim of refining these models by using disease-relevant agents is to improve their mechanistic relevance – an important consideration not only from a scientific standpoint, but also to align the spirit of the 3R's (i.e. reduction, refinement, replacement). The combination of careful experimentation, observation, as well as the application of new technologies to characterise these models has led to improved modelling systems that continue to advance our understanding of both asthma and COPD.

2. Asthma

Asthma is a condition characterised by persistent or recurrent airway inflammation, airway hyper-responsiveness, chronic airway remodelling and various degrees of reversible airflow limitation. Asthma is most commonly caused by exposure to allergens, however there are also known to be several other non-allergic triggers including cold air, exercise, stress and aspirin. The genetic and/or environmental factors that influence individual susceptibility for developing asthma are not understood. This is a significant deficit that limits our understanding of the evolution of disease manifestation, how the various triggers lead to the disease phenotype and what differentiates various patient sub-populations. As allergic asthma is the most predominant form of the disease, most in vivo models have been developed to mimic allergen-induced lung inflammation and lung function changes, which will be the focus of our review.

In allergic asthma, the process is thought to begin when inhaled allergen is detected by dendritic cells causing them to migrate to the secondary lymphatic system where they present the processed antigen to T and B cells, once specific cells are found, clonal expansion is triggered and B cell start to produce antigen specific IgE. The IgE antibodies can then bind to high affinity Fcε receptors on a variety of cell types including mast cells and basophils. Subsequent exposure to allergen leads to cross linking these receptors, causing mast cells and basophils to degranulate and release histamine, prostanooids, leukotrienes, cytokines and proteases in the airways. These mediators trigger an early asthmatic response (EAR) consisting of bronchospasm, oedema and mucus secretion. In certain individuals there is also a late asthmatic response (LAR) occurring within 24 h of allergen exposure. In addition to the EAR/LAR, inhaled antigen leads to increased airway inflammation (e.g. eosinophils, CD4+ T cells, Th2 cytokine production). These allergic events are believed to lead to remodelling of the airway structure and altered airway function (i.e. airflow obstruction and nonspecific airway hyper-responsiveness (AHR)) observed in patients.

2.1. The ovalbumin model of allergic lung inflammation

For almost 100 years, the standard approach for modelling the asthma phenotype has been to use an antigen present in egg whites, ovalbumin (OVA), to induce an allergic response in the lungs of small laboratory animals. The typical protocol starts by systemically sensitising the animals to ovalbumin absorbed onto an adjuvant anywhere from one to three times (each sensitisation usually done via intraperitoneal injections separated by at least one week). The most commonly used adjuvant is Alum or aluminium hydroxide (*Bordetella pertussis* and ricin are also frequently used), which primes the immune response towards a Th2 phenotype. This is followed by allergen exposure to the airways (usually by aerosol administration) one to two weeks after the last sensitisation. Different research groups have altered this method to optimise the specific responses they are

interested in investigating. Additionally, certain species are better than others for modelling certain aspects of this response, in part due to differences in the comparative anatomy and physiology of the laboratory animals and man (reviewed in Zosky & Sly, 2007; Stevenson & Belvisi, 2008). That said, the ovalbumin-induced allergic lung inflammation model can replicate many of the central features of allergic asthma including increased specific IgE production, oedema, mucus secretion, early and late asthmatic (bronchoconstriction) responses, a Th2-biased inflammation rich in eosinophils and nonspecific AHR. Further, these changes are sensitive to treatment with glucocorticoids and β_2 -adrenergic receptor agonists (Birrell et al., 2003; Eum et al., 2003; Wyss et al., 2007), which reflects the clinical efficacy of the standard therapies, and they have provided important insights into the nature of the allergic airway inflammation, particularly the importance of the Th2 phenotype in this disease. Unfortunately, some of these endpoints, specifically the EAR and LAR, are not routinely measured by investigators when assessing compound efficacy. Instead, AHR to methacholine challenge is the most commonly assessed physiological endpoint in these models, which, while informative, is not the only measure that will be used to judge the efficacy of a candidate compound in the clinic. This does, in our opinion, reduce the value of these models and more effort is needed to use the preclinical biomarkers that correlate to how efficacy is gauged in the clinic.

That said, there are also several shortcomings with the use of OVA itself that, some have argued, limits its utility for the purposes of current drug discovery efforts. Firstly, OVA is not an antigen associated with triggering any form of asthma in man. Secondly, attempts to sensitise animals through the respiratory tract (the likely route of sensitisation in man) have not been very successful. Thirdly, it has been proposed that chronic OVA exposure leads to tolerance and poorly maintained inflammation, possibly through the induction of T regulatory cells, which limits the ability to mimic the chronic aspect of asthma and the associated airway remodelling. There is some debate over this latter point, as investigators have reported that they are able to demonstrate some asthma-like airway remodelling after chronic ovalbumin exposure (Temelkovski et al., 1998; McMillan & Lloyd, 2004; Van Hove et al., 2009). The fact remains, however, that these models do not entirely reflect the type of inflammatory changes one would expect to see in chronic asthma as the inflammation does not worsen, but actually lessens after chronic ovalbumin exposure (Swirski et al., 2002; Van Hove et al., 2007) and the changes (i.e., inflammation and AHR) tend to resolve very quickly after the discontinuing the antigen exposures (Kumar et al., 2004). The sum of these limitations and the fact that results from the OVA model are not always indicative of what happens in the clinic, has led to the majority of academic and industrial groups switching to another antigen: the House Dust Mite.

2.2. Using house dust mite (HDM) to model asthma

In the last 5 years a great deal of work has been done to try and develop models of asthma which do not use OVA as the antigen. In particular, we will be focusing on the models using house dust mite (HDM) allergens (i.e. *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farina* (*Der f*)), although other disease-relevant aeroallergens, such as fungal allergens (e.g. *Aspergillus fumigatus*), ragweed and pollen spores, are also being routinely used and we point the reader to a review by Fuchs and Braun (2008) that cover those models in more detail.

Dermatophagoides was first identified as the source of allergen associated with house dust in 1967 and is associated with a number of allergic disorders including asthma, allergic rhinitis and atopic dermatitis (Platt-Mills, 1995). These organisms can produce thousands of proteins and macromolecules, so far over 30 of which have been shown to induce IgE antibodies in allergic individuals. The allergens associated with HDM have been identified by analysing

aqueous extracts of whole mites, nymphs, faecal pellets, eggs and spent culture media and have been divided into 19 groups. They consist of proteins and peptides with a variety of different biological functions, including proteases, binding proteins and some with functions not fully understood. The majority of the allergenicity has been attributed to group 1 (*Der p1* or *Der f1*, a cysteine protease) and group 2 (*Der p2* or *Der f2*, unknown biological function) allergens; however, there is still an incomplete understanding of the allergens present in these complex mixtures of proteins as the analysis can be affected by batch-to-batch variability, polymorphisms, as well as extraction methods (e.g. aqueous versus organic) (Thomas et al., 2002).

While the complexity of HDM extracts makes it difficult to understand the contributions of different HDM elements to allergic lung inflammation, using it to model this phenotype provides several important advantages over the OVA model. Firstly, many asthmatic subjects have raised levels of HDM specific IgE and these people have allergic events if challenged with HDM (Maunsell et al., 1968; McAllen et al., 1970). Secondly, models developed in last 5 years have demonstrated that HDM can sensitise animals via the respiratory mucosa. Thirdly, it has been suggested that giving HDM over a long time can model certain aspects of chronic asthma and associated airway remodelling.

The majority of the HDM driven models used today are based on work published by Manel Jordana's group at McMaster University in 2004. They reported that 25 μ g of HDM extract administered intranasally once a day for 10 days in Balb/C mice elicited asthma-like lung inflammation consisting of increased total IgE and HDM-specific IgG1 levels, greater numbers of eosinophils, T lymphocytes and antigen presenting cells in the airway lumen, and airway hyper-responsiveness to methacholine challenge. Additionally cultured splenocytes from HDM-exposed animals released higher levels of Th2 cytokines upon HDM re-exposure, suggesting allergen sensitisation did occur (Cates et al., 2004).

This model has allowed several groups to investigate the mechanisms underlying allergen sensitisation via the respiratory mucosa. For instance, it has been suggested that local production of GM-CSF is, in part, what prevents immunological tolerance from developing to chronic antigen inhalation (Stämpfli et al., 1998; Cates et al., 2004). The mechanism for this is not fully understood, but it has been proposed to include adjuvant-like properties that GM-CSF exhibits (Morrissey et al., 1987; Disis et al., 1996; Stämpfli et al., 1998). It had been suggested that the proteolytic activity of HDM extracts activates protease activated receptor-2 leading to increased GM-CSF production (King et al., 1998; Sun et al., 2001; Asokanathan et al., 2002; Cates et al., 2004). More recently, however, Hammad et al. (2009) demonstrated HDM-induced production of GM-CSF was dependent on Toll-like receptor 4 (TLR4) activation. Many groups have now shown the importance of TLR4 in the inflammatory response observed after HDM challenge (Hammad et al., 2009; Phipps et al., 2009; Trompette et al., 2009). Hammad et al. (2009), in a very elegant study, showed that it is TLR4 on the structural cells, and not the hematopoietic cells, which are central to the HDM-driven inflammation.

Beyond understanding mechanisms related to aeroallergen sensitisation, the use of HDM has also allowed changes associated with chronic, persistent allergen exposure to be investigated due to reported reductions in tolerogenic response to HDM. Johnson et al. (2004) investigated the effects of intranasal administration of HDM (25 μ g) for up to 7 weeks (5 days per week). They demonstrated that there were increased levels of antigen presenting cells after just 1 week of exposures, IgE, HDM-specific IgG1 and a measurable eosinophilic inflammation was present after 3 weeks, and CD4+ Th2 cells were increased after 5 weeks of HDM administration. An interesting question raised by the group was why the inflammatory response was limited, levelling off between 3 and 7 weeks exposure,

instead of progressively worsening. A clue to this answer may be in a recent paper by Gregory et al. (2009). Using a similar model they showed that inflammatory cell infiltrate and Th2 cytokines peaked after 2 weeks of exposure before attenuating and levelling off between weeks 3 and 7. There were increased numbers of T regulatory cells present in the lungs after 3 weeks of exposure, which may explain why the inflammatory changes were not progressive in response to chronic HDM administration. Johnson et al. (2004) went on to show that 5 weeks of HDM administration led to remodelling of the airways with increased mucous cell density and greater staining of contractile elements (collagen and α -smooth muscle actin) and AHR to methacholine. After 5 weeks, HDM administration was terminated to assess how quickly the changes could resolve. While the inflammatory cell infiltrate was largely resolved within 2 weeks, the airway remodelling and AHR remained elevated for at least 9 weeks (Johnson et al., 2004).

While the use of HDM extracts have improved the ability to model the persistent allergic phenotype (chronic eosinophilic inflammation, progressive airway remodelling and sustained AHR) in mice, attempts to model allergic inflammation using these extracts in rats and guinea pigs have yielded mixed results. The guinea pig was in fact the first species that was used to demonstrate that HDM extracts could be used to induce allergic sensitisation via the respiratory mucosa (Ishii et al., 1997); however, this was a very laborious process and a subsequent study relied on the use of systemic adjuvants to prime the allergic response (Yasue et al., 1999). In rats, HDM sensitisation via the airways has been shown to induce increased IgE levels and eosinophilic inflammation (Dong et al., 2003; Singh et al., 2003; De Alba et al., 2009; Jobse et al., 2009).

HDM models have provided important insights into the mechanisms underlying the initial sensitisation and subsequent induction of the allergic response. They may also provide a better understanding of the mechanisms underlying the progressive airway remodelling associated with sustained aeroallergen exposures and chronic asthma. That said we would suggest a word of caution about abandoning the OVA model and solely using the HDM model. Whilst the OVA model has been extensively studied, we currently know very little about the HDM model. We do not as yet know the role played by the major effector cells (T cells, B cells, mast cells, eosinophils, etc) or the mediators present (IgE, cytokines, lipid mediators, etc). In addition, the HDM model is often suggested to mimic allergic asthma and indeed, in some protocols (for example, Trompette et al. (2009) who demonstrated a sensitising phase is essential for a response) there is a clear allergic component. We question whether there is a clear "allergic" component in many protocols in which HDM is given daily for several weeks. We, and others, have shown that one can mimic asthma-like airway inflammation after a single topical dose of HDM in the rat (De Alba et al., 2009). Indeed we have unpublished data in the mouse in which we show an increase in white blood cells (including lymphocytes (CD4+ and CD8+)) in the lung after a single dose of HDM. These data would suggest that, initially at least, the inflammation after HDM is innate in nature. After chronic HDM administrations the response could well develop into an allergic one, and indeed various groups have reported increases in HDM specific IgE. We know however that many people in the general population have positive skin prick tests to allergens but do not suffer from asthma. Another thing to note is the fact that unlike the OVA model, no one as yet has shown EAR or LAR after HDM challenge in the model systems. It will be interesting over the next few years to see the answers to some of these questions and to see if data from the HDM model is indicative of what happens in the clinic.

2.3. Modelling severe, persistent asthma

Interestingly, while the allergic inflammation induced by HDM does respond to standard asthma therapies, specifically, glucocorticoids

(alone and in combination with a β -agonist) (Johnson et al, 2008; Ulrich et al., 2008; Wakahara et al., 2008), the combination therapy has little if any effect on AHR (and airway collagen) when administered concomitant with HDM exposures (Johnson et al., 2008). Thus, the latter feature may reflect an important step towards modelling the refractory nature of the severe asthmatic phenotype that is the predominant focus of many drug discovery efforts. Although the models using HDM may have improved clinical relevance over previous preclinical models of allergic lung inflammation, HDM models have yet to model the allergen-induced bronchoconstriction and cannot reflect the limitless AHR that occurs in severe asthmatics. Severe, persistent asthma is a poorly defined sub-population of asthmatics (~5%) whose symptoms are constant and require continuous administration of either high doses of inhaled glucocorticoids with long-acting β_2 adrenoceptor agonists and possibly treatment with oral glucocorticoids to maintain a degree of disease control (i.e., these patients may not respond to standard combination therapies) (National Heart, Lung, & Blood Institute, 1997). This inadequate definition coupled to the fact that it is highly unlikely there is a single underlying cause for this phenotype has made modelling severe asthma extraordinarily difficult.

Two models have been described in the last three years that reflect the limitless (essentially fatal) AHR associated with severe, persistent asthma. Ochur et al. (2007) reasoned that eosinophil migration, degranulation and the execution of eosinophil effector functions associated with the remodelling and dysfunction of airways in severe asthmatics (Gleich et al., 1995; Evans et al., 1997; Flood-Page et al., 2003) was a complex process that required multiple signals (Fujisawa et al., 2000; Menzies-Gow et al., 2002). Therefore they hypothesized that overexpressing both IL-5 (in T cells) and CCR3 ligands, specifically eotaxin-2 (in lung epithelial cells), in mice (I5/E2 transgenics) would mimic the extensive eosinophil degranulation observed in severe asthma, a feature that could not be replicated in other preclinical mouse models (Denzler et al., 2001). These I5/E2 transgenics displayed a marked eosinophilia and extensive degranulation similar to that observed in severe asthmatics. Additionally, the mice had epithelial shedding, luminal occlusions (consisting of cellular debris and mucous) and pronounced airway remodelling (epithelial hypertrophy, goblet cell hyperplasia, smooth muscle hyperplasia and collagen deposition). In most cases, these changes were more extensive than that observed in mice from allergen challenge models and some features were unique to the I5/E2 transgenic. In addition, I5/E2 mice had increased airways resistance and extraordinary AHR to methacholine challenge that was fatal at high doses. This phenotype could be abolished when the I5/E2 mouse crossed with the PHIL mouse, a transgenic line congenitally deficient in eosinophils (Lee et al., 2004; Ochur et al., 2007), suggesting all of these changes were dependent on the eosinophil. Interestingly, the effects of IL-5 and eotaxin-2 on eosinophil degranulation could not be replicated in vitro suggesting that an integrative setting was required where other signals could contribute to the phenotype observed in the mice.

There are several proteins housed in eosinophil granules that are suggested to contribute to the remodelling and AHR associated with severe asthma, including eosinophil peroxidase, major basic protein-1 and eosinophil cationic protein (ECP). The latter is housed in the granules of both neutrophils and eosinophils and recently, Bates et al. (2008) demonstrated that intratracheal administration of poly-L-lysine (PLL), an artificial analogue of cationic protein, could induce airway hypersensitivity to cholinergic challenges. They argued that the changes were due to the shortening of the smooth muscle banding around the airway (i.e. contracting the smooth muscle leading to a reduced luminal diameter). When they administered PLL to animals sensitised and challenged with ovalbumin, these animals died upon exposure to the low level of methacholine. Exposure to either PLL or ovalbumin alone was well tolerated by the animals; however, they reasoned that the combination of smooth muscle shortening by PLL along with allergen

provocation, which thickens the airway epithelium and increases luminal secretions, synergistically amplified the AHR. Thus, these findings may also explain the physiological mechanisms driving the fatal AHR associated with the *I5/E2* mouse and together, both studies emphasize the fact that there are likely to be layers (molecular → physiological) of complex mechanisms underlying the severe asthma phenotype.

While the actions of the eosinophil is important in severe asthma, there is a degree of control associated with individuals with high levels of eosinophils through the use of steroids and, more recently, with anti-IL-5 monoclonal antibodies (Haldar et al., 2009). Individuals with a more neutrophilic inflammation, on the other hand, are more poorly controlled. Thus, there have been recent attempts at reflecting the neutrophilic and steroid-resistant nature of the inflammation associated with poorly controlled, severe asthma. These include using chronic low dose exposures followed by a single high dose challenge of ovalbumin to mimic exacerbations (Ito et al., 2008) and overexpressing IL-13 using an adenoviral delivery system to mimic the chronic inflammation and remodelling associated with severe asthma (Therien et al., 2008).¹ In both models, steroids could still attenuate the eosinophilic and lymphocytic infiltrate, but had no effect on the neutrophil and macrophages numbers in the lavage fluid and no effect on the AHR associated with the models. Nevertheless, the inflammatory and physiological changes associated with these models are still very mild compared to those associated with exacerbations in the clinic, so the clinical relevance of these models is still questionable.

2.4. Modelling viral exacerbations of asthma

A major focus for groups in both academia and industry for many years now has been on developing models of viral-induced acute exacerbations. Exacerbations are classified events associated with an acute worsening of one's symptoms (Dougherty & Fahy, 2009). Respiratory viral infections, especially those caused by rhinoviruses (RV) are the most common causes of asthma exacerbations (50–80%) and asthma-related death in both adults and children (Johnston et al., 1995; reviewed in Singh & Busse, 2006). Severe infections spread to the lower respiratory tract where they can cause inflammation (neutrophils, monocytes and T lymphocytes), bronchiolitis, wheezing and greater airflow limitation many times requiring hospitalization. Viral infections not only cause exacerbations, but they are also common causes of bronchiolitis and wheeze in children ≤3 years of age (in particular, respiratory syncytial virus (RSV) has been implicated) (Hall, 2001; Henrickson et al., 2004; Lemanske et al., 2005), and some data support a causal link with developing asthma later in life (Sigurs et al., 2005; Lee et al., 2007; Jackson et al., 2008). The mechanism for this is unclear, but some have suggested that RSV serves as a mechanism of allergic sensitisation (Sigurs et al., 1995; Schauer et al., 2002; Sigurs, 2002) possibly through the upregulation of TLR4 receptors (Monick et al., 2003); however, there is conflicting data in this area (Stein et al., 1999). Others have proposed that individuals with a genetic predisposition for developing asthma may be more likely to develop severe respiratory infections as children because their Th1 defences are not effective, leaving them more prone to infection (Legg et al., 2003). Clearly, the mechanisms underlying the physiological changes induced by viral infections in atopic individuals are complex and incompletely understood at present.

Several groups have attempted to combine allergen and infection models to try to understand the interaction between these two responses and how they may contribute to disease susceptibility and/

or exacerbations. The majority of the work in this area has been done using RSV and the effects of RSV infections have been well characterized in several species (Prince et al., 1978; Graham et al., 1988; Hegele et al., 1993). On its own, RSV can induce acute and chronic inflammation, acute bronchiolitis, AHR and airflow obstruction (Hegele et al., 1993; Chávez-Bueno et al., 2005); however, these changes can be dependent on the strain of RSV used to infect the animals (Lukacs et al., 2006). One of the questions addressed by studies combining RSV and allergen exposures is whether RSV infection predisposes individuals to asthma or whether individuals predisposed to developing asthma are more likely to be infected. A model addressing the question of whether RSV infection in infants predisposes them to developing asthma has shown that infecting neonatal mice with RSV can affect airway inflammation and airway mechanics into adulthood (You et al., 2006). They demonstrated that the combination of RSV and OVA will lead to enhanced Th2 cytokine production, lung tissue inflammation and AHR compared to mice exposed to either OVA or RSV alone. Another study investigating whether guinea pig strains with different susceptibilities to allergen-induced inflammation had different susceptibilities of RSV infection demonstrated no such association (Bramley et al., 2003); thus, these studies suggest RSV infection may predispose individuals for developing allergic asthma. To address how RSV primes the allergic response in these animal models additional studies have investigated whether RSV infection will enhance sensitisation to allergen. Schwarze et al. (1997) found that RSV infection did in fact enhance sensitisation to ovalbumin via the respiratory mucosa. While animals exposed to ovalbumin alone did not develop airway inflammation or AHR, mice that had previously been infected with RSV did develop inflammation (consisting of eosinophils and neutrophils) and AHR, although there was no change in allergen-specific IgE or IgG1 levels. Similar findings were reported by Lukacs et al. (2001) using cockroach antigen to model the allergic response and in both studies Th2 cytokines appeared to play an important role in mediating the AHR and aspects of the inflammation. Using the chronic ovalbumin model, Tourdot et al. (2008) demonstrated that non-sensitised mice infected with RSV develop a similar degree of airway remodelling as sensitised mice (mice not sensitised or infected will not develop any airway remodelling); however, the combination of RSV infection and ovalbumin exposure did not result in enhanced inflammation or AHR. Therefore, the sum total of these studies would appear to suggest that a link between RSV and asthma exists due to RSV's ability to enhance allergic sensitisation.

There are however studies that contradict these findings. Using guinea pigs, Dakhama et al. (1999) demonstrated no changes to the inflammation or AHR induced by ovalbumin exposure in animals that had been previously or concomitantly infected with RSV. In BALB/c mice, Peebles et al. (2001) demonstrated that prior RSV infection actually attenuated the inflammation and AHR induced by subsequent ovalbumin sensitisation and challenge. Both groups acknowledged the importance of the timing of RSV infection in relation to allergen sensitisation and challenge and in the paper by Peebles et al. (2001) they demonstrated that RSV infection after ovalbumin led to greater AHR and lymphocyte infiltration in the lung. Interestingly, the same group has also shown that while concomitant RSV infection does not alter the degree of inflammation or AHR, it does prolong the AHR and lymphocytic inflammation (Peebles et al., 1999). A more recent paper by this group also shows that coordinated RSV infection and OVA challenge will lead to greater levels of mucus in the airways, possibly due to the enhanced production of IL-17 (Hashimoto et al., 2004).

Several factors may explain these anomalies in addition to the timing of the RSV infection in relation to allergen sensitisation and challenge (Robinson et al., 1997; Peebles et al., 2001). For instance, the dose of RSV (Schwarze et al., 1997; Peebles et al., 2001; Kondo et al., 2004), as well as the methods used to sensitise and challenge the animals (Schwarze et al., 1997; Peebles et al., 1999; Lukacs et al.,

¹ Interestingly, over-expressing IL-13 with the adenoviral system caused an increase in neutrophils numbers in lavage fluid, which was not a feature of the transgenic IL-13 over-expression model (Zhu et al., 1999). It is possible that the use of a viral delivery system is the reason for this difference, although the control animals did not develop any inflammatory changes after instillation of adenoviral-null vectors.

2001) can also affect the results. In addition to the various protocols used, the task of understanding the differences amongst studies is made more difficult because of the lack of relevant controls [e.g. studies that lack sham sensitised animals, animals treated with inactivated virus, or assessment of viral replication] in some of the experiments. It should also be noted that the effects of other viruses (e.g. influenza and pneumonia (Barends et al., 2004)) have been compared alongside RSV and have demonstrated different viruses may produce different effects on the allergic response.

The virus that is most commonly associated with exacerbations of asthma is RV. Unfortunately, models of RV infection had largely been limited to non-human primates for many years due to the fact that 90% of RV serotypes (major group RV, RV-1A) specifically require binding human ICAM-1 to enter cells. Some groups have however opted to use RV-1B (the remaining 10% of serotypes also referred to as minor group RV) to model RV infection because they can use the human and mouse low density lipoprotein receptor to attach and enter cells (Yin & Lomax, 1986). More recently, however, Bartlett et al. (2008) developed a transgenic mouse expressing a mouse and human ICAM-1 chimera that allowed the mice to be infected by both RV-1A and RV-1B. In this same paper, they demonstrated that co-administering RV-1B to mice sensitised and challenged with ovalbumin led to greater Th2 cytokine production and airway inflammatory cell infiltrate (especially neutrophils and lymphocytes), increased mucous production and AHR.

These models represent important steps in our ability to understand and model the contribution of viruses to both the development as well as exacerbations of asthma. However, they still do not model the severe airflow obstruction associated with acute viral exacerbations of asthma. In fact, developing such models in man has also proven to be very difficult (de Kluijver et al., 2003; Newcomb & Peebles, 2009). One possibility for moving this work further may be to use species-specific viruses (related to RV and RSV), rather than virus strains that are human pathogens. Additionally, comprehensive studies investigating the effect of viral load and timing of infection would also help to elucidate the interacting mechanisms involved in the allergic and viral responses. Further, a more complete profile of the physiological changes (i.e. EAR and LAR) associated with asthma would provide a more complete understanding of how viral infections modify the allergic responses in these animal models. Such approaches may provide a more effective route for modelling asthma exacerbation-like changes in the animals that more closely reflect the clinical situation.

3. COPD

COPD is characterized by an acceleration of the progressive airflow limitation associated with the aging process, which can leave patients disabled and in severe cases can lead to death. The disease is largely the result of years of heavy cigarette smoking, although in recent years it has been become more evident that there are also significant non-smoking causes of COPD (e.g. indoor and outdoor pollution (reviewed in Salvi & Barnes, 2009)). Like asthma, COPD is typically treated using glucocorticoids and long-acting bronchodilators (β_2 -adrenoceptor agonists or anti-muscarinics); however, these treatments are largely ineffective at attenuating the inflammation or reversing the airflow obstruction associated with the disease, which highlights the acute need for new therapies.

The deterioration of lung function is believed to be the result of structural remodelling of the lung including the narrowing of the small airways due to peribronchiolar fibrosis and luminal obstruction by inflammatory mucus exudates (obstructive bronchitis). Additionally, the parenchyma is destroyed due to proteolytic damage (emphysema) reducing the elastic drive and gas-exchange surface area of the lung, as well as the number of alveolar wall support structures that help maintain small airway patency. Although a causal link has not been

proven, free radical-driven inflammation is believed to be the force driving these pathological changes. Dogma suggests that the free radicals (e.g. reactive oxygen and nitrogen species) and reactive chemicals (e.g. aldehydes) in smoke activate resident cells in the lung, in particular epithelial cells and alveolar macrophages, to release chemotactic mediators which recruit additional inflammatory cells (neutrophils, monocytes and lymphocytes) into the lung. Chronic exposure to smoke perpetuates this response, leading to the increased production of inflammatory cytokines and degradative enzymes (e.g. proteases) as well as defects in homeostatic mechanisms (e.g. inactivation of anti-proteases, anti-oxidants and repair mechanisms). Interestingly, while all smokers develop lung inflammation, not all smokers develop COPD, suggesting that certain smokers (~25%) are predisposed for developing COPD. The factors involved in this predisposition are as yet unknown, but several hypotheses have been put forward.

The proteinase-antiproteinase imbalance hypothesis has been one of the predominant theories for explaining COPD susceptibility for nearly 50 years. The hypothesis is based on two observations: (1) a clinical study found that patients with an early-onset and familial form of emphysema were deficient for alpha1-antitrypsin, the endogenous inhibitor of neutrophil elastase (Laurell & Eriksson, 1963; Eriksson, 1965); and (2) the instillation of elastase (papain) to the lungs of hamsters led to the development of emphysema (Gross et al., 1965). Thus, the hypothesis states that individuals who are susceptible for developing COPD are likely to have deficient antiproteolytic defences, leaving the activity of proteinases unchecked (originally this activity was largely attributed to neutrophil elastase). On the basis of these observations, two in vivo models had been routinely used for many years to investigate mechanisms related to COPD and for testing compounds preclinically – the elastase and the lipopolysaccharide (LPS) lung injury models.

3.1. Models of elastase- and LPS-induced lung injury

In addition to emphysema, instillation of elastase into the lung leads to an acute alveolitis (consisting of infiltrating neutrophils and lymphomononuclear cells), mucus cell metaplasia, pulmonary edema, hemorrhage and rupture of the respiratory epithelium (Kaplan et al., 1973; Kuhn et al., 1976; Birrell et al., 2005a,b). These changes lead to alterations in lung function that are consistent with those observed in COPD patients (Birrell et al., 2005a,b). One of the major drawbacks of this model is the fact that the inflammation is transient and resolves within a week of elastase administration. Thus, it does not reflect the progressive, slowly resolving inflammation associated with COPD. An interesting feature of the model is that the emphysematous changes are progressive up until about 8 weeks, long after the inflammation has resolved, suggesting additional, unknown factors contribute to the progressive destruction of the lung. Putting those limitations aside, the mechanisms driving the inflammation and pathological changes in the model appear to be consistent to those associated with COPD. For instance, free radical stresses (Rubio et al., 2004; Ishii et al., 2005; Foronjy et al., 2006; Borzone et al., 2009), inflammatory cytokines (Lucey et al., 2002) and proteolytic damage (Kuraki et al., 2002; Houghton et al., 2006) are all essential for elastase-induced inflammation and emphysema. Additionally, statins appear to be effective in the model (Takahashi et al., 2008), whereas glucocorticoids are not (Birrell et al., 2005a,b) – findings that are consistent with clinical observations.

The biggest advantage the elastase models provide over all other in vivo models for COPD is the rapid on-set of the emphysematous destruction of the lung. Again, these changes begin to occur after just 2 weeks, progressively worsen over time, and are largely irreversible (Birrell et al., 2005a,b). For this reason, the elastase model is ideal for testing therapeutic approaches aimed at reversing or repairing emphysematous damage to the lung. The best example of such an

approach is the work published by Massaro and Massaro (1997) demonstrating the ability of retinoids to repair the parenchymal destruction induced by elastase instillation in rats. The rationale for this approach was based on the localization of retinoids in lung interstitial fibroblasts close to alveolar septal junctions during alveolar development (Maksvytis et al., 1981; McGowan et al., 1995) and the essential nature of retinoic acid receptors for proper alveoli formation (McGowan et al., 2000). These findings remain controversial as several groups have been unable to replicate the restorative properties of retinoids in similar preclinical rodent models of elastase-induced emphysema (Lucey et al., 2003; Fujita et al., 2004; March et al., 2004; Birrell et al., 2006) and data from clinical studies are lacking (Roth et al., 2006).

Because the neutrophil is the largest source of neutrophil elastase, it was regarded as the central player driving the pathologies associated with COPD for many years. As such, many early broad spectrum anti-inflammatory drug discovery efforts were aimed at inhibiting neutrophil infiltration and the LPS acute lung injury model was ideal for these studies. LPS is a bacterial endotoxin that is present in cigarette smoke and, when instilled into the airways, it can elicit a pronounced neutrophilic inflammation that peaks between 6–24 h post-administration (Ferretti et al., 2003). Chronic administration of LPS has also been shown to lead to airway remodelling, emphysema and altered lung function (Stolk et al., 1992; Savov et al., 2003; Brass et al., 2008). Although robust, the clinical relevance of this model is questionable in that the inflammation it evokes is inhibited by glucocorticoids (Birrell et al., 2005a,b) and repeated LPS administration attenuates the neutrophilic inflammation (i.e., multiple administration of LPS induces immunological tolerance (Brass et al., 2008)); therefore it does not reflect the progressive, slowly resolving and steroid-insensitive inflammation that is a hallmark of COPD. While the LPS model is still commonly used as a mechanistic model for investigating mechanisms regulating TLR-mediated neutrophilia and acute lung injury, it is no longer routinely used as a “disease” model for assessing prospective COPD therapies for the reasons just described.

3.2. Using cigarette smoke to model COPD-like changes

Over the last 10 years, in vivo models of cigarette smoke-induced lung inflammation and lung damage have become the preferred preclinical system for investigating mechanisms related to COPD. The biggest advantage these systems offer over other models for COPD is the ability to use the primary disease-causing agent to model several key features of the disease in small animals. There are over 4000 chemicals and $>10^{15}$ free radicals in every puff of smoke (Pryor and Church, 1993); thus, single stimuli such as elastase and LPS are unlikely to replicate the multifaceted response to smoke. Although the cigarette smoke has been used for decades for modelling lung diseases in small animals (primarily in studies investigating the link between smoke and lung cancer (Mertens, 1930)), it was not until 1990 that systems using cigarette smoke for modelling COPD began in earnest. In that year, Wright and Churg (1990) demonstrated chronic cigarette smoke exposure in guinea pigs led to progressive emphysematous changes that were associated with changes in lung function consistent to those observed in human emphysema patients. Several groups have since confirmed these findings in several species and have shown that in addition to emphysema, these models can also reflect mucus cell metaplasia and epithelial hypertrophy in the central airways as well as the fibrotic remodelling of the smaller airways, both of which are consistent to the changes observed in COPD patients (Bartalesi et al., 2005; Stevenson et al., 2007; Wright et al., 2007).

These pathophysiological changes are again believed to result from the progressive, low-grade inflammation chronic cigarette smoke elicits. The inflammatory response to smoke exposure is not as pronounced as the response to either LPS or elastase. In addition, it

appears to have two phases – a transient acute phase dominated by neutrophilic infiltrate peaking during the first week and a progressive chronic phase composed of neutrophils, macrophages and lymphocytes infiltrating the lungs starting after 1 month of exposures (D'hulst et al., 2005; Stevenson et al., 2007). There does appear to be some overlap in the mechanisms driving the inflammation during both phases (Pemberton et al., 2005; Morris et al., 2008); however, there is also some evidence to suggest that there may be important differences that require further elucidation (Maes et al., 2006; Wan et al., in press). In addition, to being progressive, the inflammation is very slow to resolve. In a study looking at the effects of increasing duration of smoke exposures in rats, Stevenson et al. (2007) showed that after 6 months of exposures that several inflammatory indices (although lower) were still present 2 months after exposures had been stopped. In (C57BL/6) mouse models, chronic smoke exposure causes B cell follicles to develop progressively over time (D'hulst et al., 2005; van der Strate et al., 2006). These resemble the neo-germinal centers found in the lungs of COPD patients, which has led some to propose that COPD becomes an autoimmune disease in the later stages and independent of cigarette smoke exposure (Agustí et al., 2003; Hogg et al., 2004); however, while the presence B cell follicles in the mouse models do correlate with the degree of emphysema, there is no evidence that the pathologies in animal smoking models can continue to develop after smoke exposures have stopped (van der Strate et al., 2006). Further, the inflammation in these small laboratory animal smoking models do not appear to be affected by treatment with glucocorticoids (Marwick et al., 2004; Marwick et al., 2009); thus, these models appear to effectively mimic the progressive, low-grade, slowly resolving and steroid-insensitive inflammation associated with COPD.

Data from animal models of cigarette smoke exposure provide the strongest evidence for a causal link between inflammation and the pathologies associated with COPD. Ofulue et al. (1998) demonstrated that smoking-induced emphysema was completely inhibited in rats treated with an anti-macrophage antibody. This confirmed a finding from a previous study using genetically modified mice that showed MMP-12 (macrophage metalloelastase) was the principle mediator driving the emphysematous destruction of the lung precipitated by cigarette smoking (Hautamaki et al., 1997). Since these early studies, several groups have reported the importance of several pathways including cytokines (e.g. TNF- α , IL-1 β , IL-18, IFN- γ), chemokines (e.g. CXCR2, CXCR3, CCR5, CCR6) and enzymes (serine proteases, matrix metalloproteinases, p38 MAP kinase, phosphodiesterase 4) involved in regulating the inflammatory response and subsequent lung damage elicited by cigarette smoke exposures (see Churg et al., 2008 for a complete review). The mechanisms involved in the initial response to smoke are at present unknown, although pathways (e.g. nuclear factor E-2 like 2 (Rangasamy et al., 2004; Foronjy et al., 2006)) and receptors (e.g. TLR4 (Maes et al., 2006; Doz et al., 2008)) associated with the response to free radical stress have been implicated. Whether findings from these models will translate into new clinically efficacious therapies remains to be seen.

There is also some indication that the lack steroid-efficacy in the models is due to mechanisms induced by free radical stress thought to be clinically relevant (Marwick et al., 2004; Ito et al., 2006). There are, however, mechanisms that have demonstrated anti-inflammatory efficacy in the preclinical models that have not been effective clinically. For instance, mice lacking TNF- α receptors have been shown to be partially protected against smoking-induced inflammation and emphysema (Churg et al., 2004); whereas anti-TNF- α monoclonal antibodies have not demonstrated a benefit in the clinic (van der Vaart et al., 2005; Rennard et al., 2007). The reason for this discrepancy between the model and the clinic is unclear, but we propose that it may be that TNF- α is critical for the initiation of the response to smoke (acute phase) and may become less important over time (the latter obviously cannot be assessed with a knockout

mouse). One way to test this hypothesis would be to administer a mouse anti-TNF- α monoclonal antibody therapeutically after the chronic inflammatory response to smoke exposures had already been established (>1 month). This example highlights an important point regarding the practical application of these smoking models for assessing potential therapeutic interventions. Because these models require a long time (~4–6 months) to generate pathologies and functional changes consistent to those observed in COPD patients, several groups use an acute (<1 week) model of smoking-induced lung inflammation as primary models to examine the efficacy of prospective therapies. As was previously mentioned, there does appear to be certain mechanisms that will be important in both the acute and chronic phase of the response to smoke exposures; however, the effects of compounds should always be confirmed in more chronic models, ideally under therapeutic dosing regimens, for a more complete assessment a compound's potential for inhibiting the inflammation, pathologies and physiological changes associated with chronic smoking.

One of the limitations of the smoking models is the fact that there is no *one* model of smoking induced lung inflammation and lung damage. Several groups use different delivery systems (nose-only versus whole-body), different species or strains, different cigarettes, different components of the smoke (mainstream versus sidestream) and different doses of smoke (typically assessed as milligrams of particulate matter per cubic meter). This can make extrapolating findings from one group to the next difficult. That said, while some differences may exist in the extent of the changes elicited by smoke exposures, many groups using different systems have generated similar results (e.g. Vlahos et al., 2006 and Morris et al., 2008). Additionally, while these smoking models can replicate many of the key features of COPD in small animals, the changes are very mild compared to those observed in man. Unfortunately, (as with the asthma models) lung function is not routinely assessed in a meaningful way. Instead, it is all too often replaced by measuring histopathological changes, which is not an endpoint used to assess efficacy in the clinic. This is partly to do with the difficulty in measuring lung function in mice, which is the species most groups use, as well as the mild nature of the structural changes; however, technologies have improved considerably in recent years and more work in this area is needed.

The pathologies and lung function changes associated with these models would most likely constitute mild (GOLD 1) COPD (Churg & Wright, 2007; Rabe et al., 2007). Unfortunately, most cases of COPD are not diagnosed until the disease has become severe (GOLD 3) and it is not clear whether, in addition to the physiological differences, the mechanisms driving the disease also differ as the disease progresses (Agustí et al., 2003; Hogg et al., 2004). One of the major differences between early- versus late-stage COPD patients is the incidence of disease exacerbations, which become more frequent once a patient's lung function drops below a certain threshold (forced expiratory volume in 1 second is <50% predicted) – this is also when their disease is classified severe (GOLD 3).

3.3. Modelling exacerbations of COPD

Acute exacerbations in COPD patients, as in asthmatics, are a major cause of hospitalization and death. They are also known to contribute to the accelerated lung function decline and disease progression (Donaldson et al., 2002; Makris et al., 2007). For all of these reasons, several groups have begun to challenge animals exposed to smoke with agents known to cause exacerbations (e.g. bacteria and viruses) in an attempt to model the pathologies and physiological changes associated with late-stage COPD. In addition, as with the asthma models, these systems may further our understanding of how smoke exposure affects the innate responses to bacteria/viruses (and vice versa) and the mechanisms that may underlie acute exacerbations.

Viruses are considered to be the most important initiators of COPD exacerbations. One of the first studies combining virus and cigarette smoke was by Meshi et al. (2002) who demonstrated that latent adenoviral infection enhanced the inflammation and lung pathology (i.e. emphysema) compared to that of cigarette smoke exposure alone. In this study the effects on the macrophage and neutrophil infiltrate was additive and each agent recruited a distinct population of T cells to the lung. While it did appear that adenovirus infection worsened the emphysema, these changes were only modest; however, this study represents an important first step in using the combination of smoke exposure and viral infection to cause greater damage to lung that may more accurately reflect the degree of damage observed in the lungs of COPD patients with advanced disease. More recent attempts have looked at the effect of viral infection after smoke exposure. Gualano et al. (2008) assessed the impact 3–10 days of cigarette smoke exposure had on the response to a subsequent influenza infection using mice. They hypothesized that smoke exposure would increase the level of inflammation in the lung leading a more rapid clearance of the infection; however, the results did not support this thesis. Instead it was observed that animals exposed to smoke and infected with virus developed a greater level of lung inflammation. Rather than having increased viral clearance, viral titres were elevated in smoke exposed mice after 3 days. The authors attributed to possible smoking-induced alterations in how the T cells responded to the influenza infection. Observations that cigarette smoke impairs anti-viral responses has also been observed in human preclinical in vitro models (Modestou et al., 2010). A similar finding was reported by Robbins et al. (2006) in a chronic smoking model (3–5 months), but using a different strain of mice and influenza. In this study, the effect on smoke exposure on the response to infection depended on the dose of influenza. After 3 – 5 months of smoke exposure animals infected with a low dose of virus (that produced no clinical symptoms) had less lung inflammation and enhanced viral clearance (the latter was attributed to a heightened type IFN response) compared to control mice infected with influenza only. On the other hand, animals exposed to smoke and infected with a high-dose of virus (that produced clinical symptoms) progressively worsened and nearly 35% of those mice died 7 days after the infection. The combination of smoke and high-dose of influenza also led to greater BAL inflammation, but there was no change in lung tissue inflammation compared to control mice infected but not exposed to smoke. The underlying mechanisms for the worsened symptoms associated with combining infection with smoke exposure remain unclear as there were no differences in viral clearance, T cells populations, or the secondary antibody responses upon re-infection. That said, from these three studies it is clear that combining cigarette smoke exposures with a single viral infection will likely lead to enhanced inflammation and lung pathologies. Next steps may include investigating the effects of multiple infections in addition to chronic smoke exposure on lung inflammation, structure and function.

There has been some progress moving in the direction of the last point, but rather than using live virus, some groups have opted to use components of viruses known to trigger the activation of pathogen-associated molecular pattern (PAMPs) pathways, such as polyinosinic: polycytidylic acid (poly-IC). Poly-IC is a synthetic version of the dsRNA present in some viruses and has been shown to activate Toll-like receptor 3 (TLR3). In a study by Kang et al. (2008), two strains of mice were exposed to cigarette smoke for 4 weeks and during the last two weeks of the exposures, the mice were also administered 4 doses of poly-IC. The combination of smoke and poly-IC led to a heightened inflammatory response, airspace enlargement and evidence of fibrotic remodelling of the airways (Kang et al., 2008). They also found similar results using influenza in combination with smoke. These types of pathological changes are typically only observable after several (4–6) months of smoke exposures; thus, this provides further evidence that combining these stimuli will accelerate the development of COPD-like

changes occurring in the lungs of small laboratory animals. It will be interesting to see whether these changes can become progressive with increasing duration of smoke exposure and whether they resolve when the poly-IC challenges or smoke exposures are stopped. Further, the effects on lung function also need to be characterised in these systems because these are important preclinical correlative endpoints that relate to those used to assess a patient's condition.

In addition to using virus and virus-associated molecules, infecting small animals with bacteria has also been used to model COPD acute exacerbation-like changes. Several types of bacteria are associated with COPD exacerbations including *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and nontypeable *Haemophilus influenzae* (NTHI), the latter being the most common isolated from the airways of COPD patients. Recently, it was reported that after 8 weeks of cigarette smoke exposures, infection with NTHI led to enhanced airway inflammation, although there was no assessment of COPD-related pathologies (Gaschler et al., 2009). Exposure to cigarette smoke skewed the profile of inflammatory cytokines produced after infection with NTHI and enhanced the clearance of NTHI in the lung. The same group had previously shown that smoke also altered the response to infection with *Pseudomonas aeruginosa*, an opportunistic infection that occurs in late-stage COPD patients. In animals exposed to smoke and infected with *P. aeruginosa* there was a worsened clinical score and a heightened inflammatory response, but in this study they attributed these changes to smoke exposures reducing the clearance of the infection from the lung (Drannik et al., 2004). The reasons for the differences observed in these two models are unclear at present, but underscores the complexity of combining cigarette smoke exposure with human pathogens to model these acute events, including the type of bacterium used as well as the virulence of the chosen strains (Chin et al., 2005). In addition to being a major cause of exacerbations, bacterial colonization of the lung is also likely to affect disease progression and can occur in patients with stable COPD (i.e. those who have not experienced an exacerbation). Developing models of colonization has been difficult because most of the human pathogens associated with exacerbations of COPD are typically cleared within 24 h after instillation into rodent airways. As we previously suggested, moving towards using more species-specific pathogens may allow investigators to mimic these types of infections more effectively; however, a major caveat to this approach will be to determine what the comparative differences are in the immunological responses to different pathogens in different species.

3.4. Using mouse genetics to understand mechanisms underlying COPD susceptibility

Although there are comparative differences in genetics, anatomy and physiology, the use of laboratory animals obviously allows investigators to test hypothesis about the causal mechanisms underlying disease in ways that cannot be done in man. The mouse has become the species of choice for most of these studies because of the availability of molecular tools, the variety of in-bred strains with different phenotypic traits and, most importantly, the entire mouse genome has been sequenced and is readily manipulated to generate genetically modified mouse models.

The mouse has also been very important for our understanding of the pathogenesis of COPD. There are several naturally occurring mutant mouse strains (e.g. Tight skin, beige, pallid), gene-knockout mouse lines (e.g. retinoic acid receptor- $\gamma^{-/-}$, surfactant D $^{-/-}$) and transgenic mouse lines overexpressing certain mediators (e.g. IL-13, IL-18, IFN- γ) that develop airspace enlargement (reviewed in Mahadeva & Shapiro, 2002). In some cases, the emphysema-like changes are the results of abnormal development (e.g. Tight skin, beige and retinoic acid receptor- $\gamma^{-/-}$ mice); however, in all cases the phenotypes shed some light on the mechanisms controlling alveolar development and deterioration. Furthermore, knockout and transgenic mouse models have yielded insights into mechanisms directly

involved in mediating the inflammation and lung pathologies induced by smoke exposures (e.g. Nrf2 $^{-/-}$ and MMP-12 $^{-/-}$ mice).

While these monogenetic mouse models have provided insights into COPD pathogenesis, susceptibility for developing complex syndromes such as COPD are more likely to be the result of a combination of allelic variants that leave an individual more susceptible to the effects of cigarette smoke exposure. There have been several groups who have started to compare the degree of cigarette smoke-induced lung damage amongst different strains of mice in an attempt to understand the genetic factors that may underlie COPD susceptibility. The earliest approaches compared the effects of smoke exposure in a few mouse strains with differences in specific molecular phenotypic traits – i.e. different levels of α 1-antitrypsin (Cavarra et al., 2001), different antioxidant responses (Bartalesi et al., 2005) and different major histocompatibility complex haplotypes (Guerassimov et al., 2004). Currently, there are ongoing studies using more sophisticated molecular genetics technologies in an attempt to link the genetic variation amongst mouse strains to the differences in their susceptibility to smoking-induced lung pathologies in hopes of understanding more about the polygenetic mechanisms underlying COPD (Shapiro et al., 2004). Such studies will hopefully lead not only to new insights into common mechanisms associated with COPD pathogenesis, but may also provide more information that may help define distinct COPD patient sub-populations.

3.5. Modelling systemic co-morbidities of COPD

In addition to the progressive airflow obstruction, COPD patients often suffer from additional conditions that can impact on their prognosis. Cardiovascular disease, lung cancer, pulmonary hypertension, diabetes, osteoporosis and cachexia are common co-morbidities that patients may suffer from in addition to COPD. These conditions cannot only impact on the functional capacity of the patients limiting their mobility and quality of life, but can also be a direct cause of death. It is not understood whether these co-morbidities are independent co-existing conditions (as a result of the advanced age or smoking history of the patient) or a consequence of the patients' COPD. In either scenario, it is generally agreed that the inflammation associated with smoking and COPD will contribute to and worsen these co-morbidities. To account for the impact these comorbid diseases have on patient prognosis, recent clinical studies (e.g. TORCH and UPLIFT) have begun to use all-cause mortality as an endpoint to assess the efficacy of therapeutic approaches (reviewed in Sin et al., 2006). Additionally, drugs that may impact on the co-morbidities (e.g. statins) may also provide an important therapeutic benefit for COPD patients and are currently being trialled clinically (reviewed in Barnes & Celli, 2009).

To further our understanding of the important role co-morbidities may play in COPD pathogenesis, investigators are using the rodent smoking models to mimic some of the pulmonary and systemic co-morbidities associated with COPD. For instance, several groups have demonstrated that chronic cigarette smoke exposure can induce pulmonary hypertensive-like changes (Wright & Churg, 1991; Nadziejko et al., 2007). These models are now being used to assess the efficacy of compounds against both the hypertensive- and COPD-like changes in the same animals (Sussan et al., 2009). Other groups have started to focus on examining the effect chronic cigarette smoke exposure has on systemic inflammation and how it leads to changes in peripheral skeletal muscle fibre-type composition as well as muscle atrophy in mice (Gosker et al., 2009; Tang et al., 2010). These models may provide new insights into the mechanisms associated with this systemic feature of the disease that is associated with a poor prognosis (Schols et al., 2005).

Additionally, new models are also being developed to investigate the link between COPD and cardiovascular co-morbidities. For example, a clinical association between the degree of emphysema and arterial stiffness has been established (McAllister et al., 2007). Some investigators have now begun to use animal models to further

investigate these associations and genetic models of atherosclerosis (e.g. ApoE^{-/-} and Ldlr^{-/-} mice exposed to a Western diet) have also demonstrated such a link exists (Golovatch et al., 2009). The fact that cigarette smoke has been shown to induce atherosclerosis-like changes in ApoE^{-/-} mice through different pathways and independent of a high fat diet (Stolle et al., 2008) suggests a logical next step would be to determine whether these models can interact to determine produce enhanced lung pathologies and reduced functional capacity.

These models mimicking the systemic aspects of COPD represent an exciting new approach for investigating the mechanisms underlying the pathophysiology of the disease. Investigating the interaction between cigarette smoke, extrapulmonary physiological changes, and changes to lung structure and function may expand the number of therapeutic options and lead to novel approaches that can be used to help manage the disease.

4. Next steps for modelling allergic asthma and COPD

Over the last decade, there have been significant improvements in the methods for modelling allergic asthma and COPD in small animals; however, it is clear that no one model will provide all the answers. For asthma, these include the use of more clinically relevant antigens and methods of sensitisations as well as the ability to model fatal AHR that is associated with the severe form of the disease. However, there is still a need to develop models of severe asthma that can reflect not only severe AHR to methacholine challenge, but also severe bronchospasm as a result of allergen or viral exposure that is not effectively attenuated with glucocorticoids or β -agonists.

For COPD, using cigarette smoke to model the disease has been established as the primary model used by most investigators. These models can be used to mimic the pathological and physiological changes in the lung as well as some of the systemic changes associated with COPD. That said, the severity of these changes in the models are mild compared to the clinical manifestation of the disease and changes in lung function are not routinely assessed. New models combining cigarette smoke exposure with systems mimicking extra-pulmonary conditions commonly associated with COPD may lead to alterations in physiology that more accurately reflect the clinical disease.

The progress that has been made is encouraging, but the challenge is still great. In addition to building on the work reviewed here, next steps should include a concerted effort to use these models to understand more about the interaction between polygenetic determinants, baseline physiology and early-life events that may underlie the susceptibility to allergen- and cigarette smoke-driven changes in physiology. Further characterisation of the interaction between viral and/or bacterial infections and these [allergen- or smoke-driven] models, may also provide improved models of severe respiratory disease (Fig. 1). Additionally, routinely assessing preclinical correlates to the parameters measured in the clinic is essential to our understanding of how these mechanisms may translate. Integrating the knowledge gained through in vivo models in parallel with clinical observations and studies conducted using clinical specimens will hopefully lead to further insights into how we understand, define and treat these chronic respiratory diseases.

5. Uncited references

Church and Pryor, 1985
Ford et al., 2009
Leme et al., 2009
Noble et al., 1997
Wright et al., 2008

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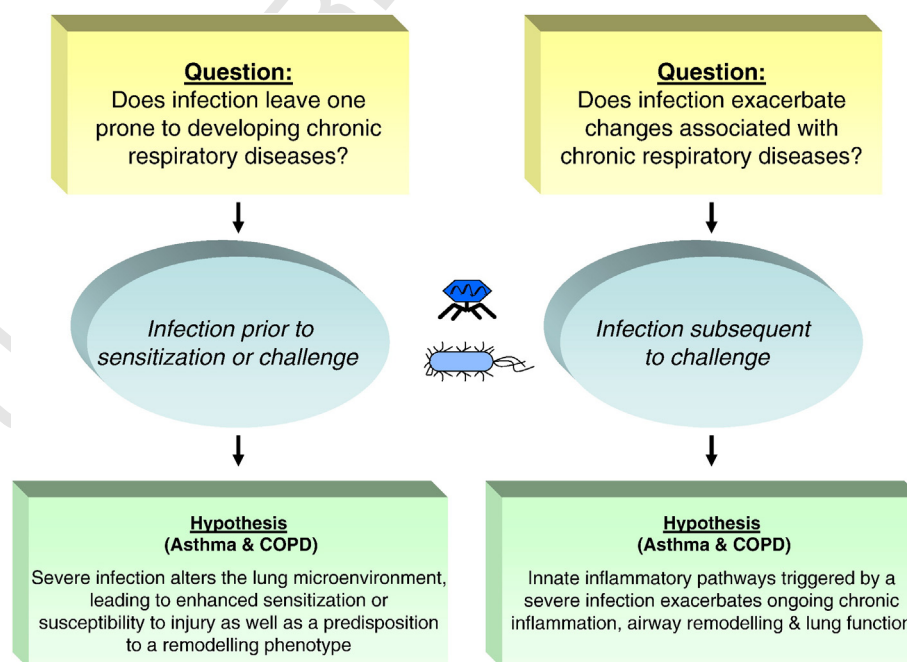


Fig. 1. How do infections contribute to asthma and COPD pathogenesis? This schematic poses two questions and related hypotheses explaining how infections may contribute to asthma/COPD susceptibility and disease progression. These ideas can be initially tested in preclinical in vivo models by altering the timing of infections and challenges to “disease”-inducing agents (i.e. allergen or cigarette smoke).

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