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STATE-OF-THE-ART REVIEW

Animal models of asthma

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Clinical and Experimental Allergy

Summary

Animal models of asthma are a tool that allows studies to be conducted in the setting of an intact immune and respiratory system. These models have highlighted the importance of T-helper type 2 driven allergic responses in the progression of asthma and have been useful in the identification of potential drug targets for interventions involving allergic pathways. However, a number of drugs that have been shown to have some efficacy in animal models of asthma have shown little clinical benefit in human asthmatics. This may be due to a number of factors including the species of animal chosen and the methods used to induce an asthmatic phenotype in animals that do not normally develop a disease that could be characterized as asthma. The range of animal models available is vast, with the most popular models being rodents (inbred mice and rats) and guinea-pigs, which have the benefit of being easy to handle and being relatively cost effective compared with other models that are available. The recent advances in transgenic technology and the development of species-specific probes, particularly in mice, have allowed detailed mechanistic studies to be conducted. Despite these advances in technology, there are a number of issues with current animal models of asthma that must be recognized including the disparity in immunology and anatomy between these species and humans, the requirement for adjuvant during senitization in most models, the acute nature of the allergic response that is induced and the use of adult animals as the primary disease model. Some larger animal models using sheep and dogs have been developed that may address some of these issues but they also have different biology from humans in many ways and are extremely costly, with very few probes available for characterizing allergic responses in the airway in these species. As research in this area continues to expand, the relative merits and limitations of each model must be defined and understood in order to evaluate the information that is obtained from these models and to extrapolate these findings to humans so that effective drug therapies can be developed. Despite these issues, animal models have been, and will continue to be, vital in understanding the mechanisms that are involved in the development and progression of asthma.

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Why are animal models necessary?

As research into asthma has expanded, the number of risk factors, processes and mediators that have been identified has increased exponentially, leading to the realization that asthma is a complex and heterogeneous disease that is driven by a variety of processes that occur at cellular [1], molecular [2] and genetic [3] levels. Ideally, in order to further our understanding of the mechanisms of this disease and to identify critical pathways, and hence targets for drug therapies, in-depth studies should be conducted on human asthmatics. However, for obvious ethical reasons the types of experiments required to dissect accurately the mechanisms that are involved at cellular and molecular levels are not possible in humans. For example, a mediator may be identified as deficient or up-regulated in asthmatics compared with healthy controls from lung biopsies of fatal asthma attacks or in bronchial epithelial cells taken under anaesthesia [4]. Having identified this mediator, it is necessary to

determine whether it is important in the pathogenesis of the asthmatic condition or whether it is simply a by-product of the inflammatory processes that are generally thought to drive disease progression. In the absence of being able to conduct the required experiment in human asthmatics due to ethical and logistical limitations, a system for modelling the process needs to be developed to determine the importance of the identified mediator.

For physical processes, such as describing patterns of flow limitation in the airways, it may be possible to use a mathematical modelling system [5]. However, for immunological processes at a cellular or molecular level, a biological model is required. *In vitro* modelling systems that utilize standard cell lines or cells obtained directly from human asthmatics are used extensively in studies on asthma and have been particularly useful in understanding cell signalling [6], cellular responses under T-helper type 2 (Th-2) conditions [7] and wound repair [8]. While *in vitro* models are useful from this point of view, they are somewhat limited by the fact that they are far removed from the *in vivo* situation, which is characterized by a complicated interplay between physical and chemical processes and involves numerous structures and cell types within the lung and throughout the body. Attempts have been made to mimic the structural environment of cells in the airway through the use of a three-dimensional *in vitro* model of the cells in the airway [9]. Manipulation of the cytokine milieu during cell culture can also be used to model the environment in the airway for *in vitro* experiments [10]. However, such artificial environments are difficult to standardize and require prior knowledge of the dynamics of the immune response and the complicated interplay between cells in the airway to design experiments effectively. Consequently, the only system that is available for modelling *in vivo* processes of human disease, and the one that provides the most holistic approach to understanding the pathobiology of asthma, is the animal model.

Animal models of asthma have been used for over 100 years [11] and, while, as with any model of disease, the link between the processes observed in the animal and humans needs to be carefully considered, they are an ideal vehicle for identifying and testing mechanisms linked to the development of the asthmatic phenotype. Animal models are particularly useful for mechanistic studies whereby a mediator or process, once identified, can be manipulated, by antagonism, suppression or up-regulation, to examine its role and importance in leading to the development of abnormalities that are characteristic of asthma such as inflammation, remodelling and airway flow limitation. There are numerous examples of processes and mediators that have been identified in animal models and are now known to be critical in the development and progression of disease in humans. A classic example of this is the identification of the relative importance of Th1 and Th2 in mouse models of allergic airway inflammation [12]. Such studies in mice have identified the importance of the Th-2 phenotype in the progression of allergic disease and in particular the importance of the cytokines IL-4 [13–16], IL-5 [17–19] and IL-13 [20, 21] in the perpetuation of allergic inflammation and the development of increased responsiveness of the airways.

The same rationale for the use of animal models in mechanistic asthma research can be applied to drug discovery and development. For example, having identified a pathway that appears to be critical in the progression of an asthmatic phenotype in an animal model, drugs that may modify this pathway and prevent disease progression can be developed. The range of mediators that have been identified as potential drug targets in asthma is vast and, when combined with the number of drugs that may alter the identified pathway, the decision to begin clinical trials in humans may be hampered by the expense associated with testing such a large number of potential therapies. In order to avoid the problem of deciding which drugs are worthy of a full-scale clinical trial, a screening process is required to identify those that are most likely to have therapeutic benefit. Again, the most efficient way to develop a drug-screening process is to develop a model of the system that is of interest. *In vitro* models may be used to determine the efficacy of a drug in modifying a particular process at a cellular level [22]. However, *in vivo* animal models are the most effective tool for studying the effect of a drug as they involve intact immune and respiratory systems. Animal models not only allow testing of the efficacy of a particular drug, they also allow a primary estimate of the toxicity of the drug, which can then be used as a starting point for a human clinical safety and efficacy trial. An example of this is the long-standing use of the guinea-pig as a vehicle for testing the efficacy of drugs for immediate hypersensitivity reactions such as those induced by exposure to toxic particles and those associated with occupational asthma [23]. Similarly, a number of potential drug therapies including montelukast [24], integrin antibodies [25] and tryptase inhibitors [26] have all been tested for their efficacy in sheep models of asthma.

Clearly, animal models of asthma represent a useful system for understanding disease pathobiology and for developing and testing potential drug therapies. However, the interpretation of the results from such models and the extrapolation of these results to human asthmatics are highly dependent on the outcome of interest and the species of animal chosen.

Relative merits of different species

In attempting to model any system, in this case a disease process, the first decision to make is what element/s of the

system one would like to model. A majority of animal models of asthma have targeted the 'classic' Th2 asthmatic phenotype, which is characterized by high levels of antigen-specific IgE, airway inflammation dominated by eosinophils and a pattern of Th2 cytokines including IL-4, IL-5 and IL-13 [27, 28]. The appropriateness of this paradigm has been reviewed a number of times elsewhere and is not the purpose of the present discussion. However, given that a majority of studies, historically, are based on this phenotype this review will focus on the capacity of animal models to develop phenotypic characteristics that have traditionally been associated with Th2-driven allergic asthma. No laboratory animal is known to spontaneously develop a disease with characteristics that can be considered to be asthma [29]. There are some examples in the animal world that are similar to asthma; cats may develop a bronchial disease that is similar to human chronic asthma [30], horses may develop a neutrophil dominated airway disease known as heaves that has some of the hallmarks of asthma [31], but may be more closely related to chronic obstructive pulmonary disease, and both sheep [32] and dogs [33] are known to have a natural susceptibility to some allergens. Consequently, the development of most animal models of asthma involves a process of sensitizing the animal to an antigen of interest and subsequently challenging the airways in order to elicit an allergic response. It is well known that the responses of the airways to such a protocol can differ substantially between species. This is due to a number of reasons including the method of sensitization [34], the antigen used [35] and species-specific differences including anatomical, physiological and immunological responses [36]. This variability is also apparent within a single species such as the differences in physiological and immunological responses observed between strains of mouse for a single protocol [37, 38]. The species chosen and method of 'creating' the asthmatic phenotype are highly dependent on the particular aspect of the disease that is of interest with some species or strains being better for modelling particular characteristics of asthma than others. The relative merits of a number of species that are used as models of asthma are discussed below.

Mice

Mouse models of asthma have become, by far, the most popular animal for modelling allergic responses in the airways. Mice are an ideal species for the study of most diseases as we have a detailed understanding of their genetics [39], it is easy to manipulate outcomes using transgenic technology [40], there are numerous commercially available mouse-specific probes for studying allergic outcomes and they are relatively cheap, allowing large studies to be conducted. Mice are easily sensitized to a number of antigens, to which they are not normally exposed, including ovalbumin (OVA) [41], which is the most popular, and a number of recognized human allergens such as house dust mite (HDM) [35, 42], cockroach antigens [43], *Aspergillus fumigatus* [44] and ragweed extracts [45]. Sensitization and subsequent challenge with these antigens result in a clearly defined Th-2 type response in the lungs, with the level of antigen-specific IgE [46], eosinophilia [47] and responsiveness of the airway to bronchoconstricting agents [37] varying considerably between strains. This simple difference in response between strains for a given antigen senitization and challenge protocol can be seen as an advantage of mice as models of asthma as it allows the identification of cellular [38] and genetic [37, 48, 49] mechanisms of inflammation and airway responsiveness. The rapid expansion in transgenic technology in recent years makes mice ideal for mechanistic studies, whereby a single molecular pathway can be switched off, suppressed or up-regulated [40] in order to understand the importance of this pathway in the development of the asthmatic phenotype . Such studies in mice have highlighted the importance of the cytokines IL-4 [50], IL-5 [51] and IL-13 [52], which are thought to be critical in Th2-driven allergic reactions in the airways in a large portion of human asthmatics. The identification of the importance of these cytokines and mediators in the allergic process, the bulk of which have been studied extensively in mice, has led to the development of a number of potential therapeutic targets [53]. However, the therapies that appeared to have efficacy through these pathways in mouse models of asthma have not been successful in human clinical trials [53].

The plethora of mechanisms that have been identified as potentially important in the pathobiology of asthma and the vast array of tools available for studying mice seem to make them the most appropriate model for this disease. There are, however, a number of issues that have been identified, and debated [54, 55], regarding the appropriateness of mice as a model for human asthma [56–60]. The inflammatory response in sensitized mice to antigen challenge, depending on the dose of antigen used, usually results in a massive influx of inflammatory cells, dominated by eosinophils, into the airways [61]. In these cases, eosinophils may represent up to 60% of the cells obtained from the bronchoalveolar lavage (BAL) [41] and the histological pattern of inflammation is more reminiscent of an allergic alveolitis [58] rather than the asthmatic phenotype that is being modelled (Fig. 1). From an immunological point of view, there is considerable controversy regarding the importance of eosinophils in mouse allergic airway responses as it is known that eosinophils rarely degranulate in mouse models of asthma [62] whereas human eosinophils readily degranulate [63]. One other potential problem with mice, and rodents in general, is the pattern of mediators released

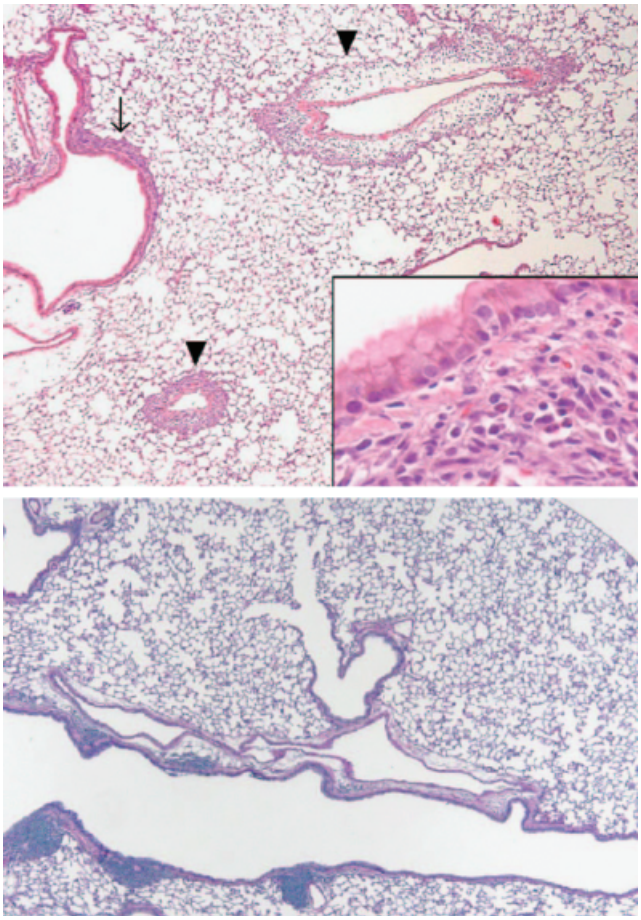

Fig. 1. Histological slides showing the extreme peribronchial (↓ , inset) and perivascular (▼) inflammation in a standard mouse model of asthma (top panel) that is more akin to allergic alveolitis than asthma. The bottom panel shows a mouse model of asthma where the mouse is exposed to repeated low-dose challenges over a period of weeks, resulting in a more moderate level of inflammation that is localized in the main conducting airways [69] (redrawn from Collins et al [155], with permission from Elsevier).

by mast cells. It is well known that rodent mast cells release serotonin, which is not thought to play a role in human asthma [29]. Such discrepancies must be taken into account when extrapolating data from animal models to humans.

Similarly, if the physiological response of the asthmatic lung is broken down into the immediate responses to antigen exposure and non-specific changes in lung physiology, as indicated by airway hyperresponsiveness (AHR) to bronchoconstricting agents, then mice are known to be deficient as models in a number of areas. Following inhalation of an allergen, the asthmatic lung often develops an early-phase response (EPR) that is characterized by acute bronchoconstriction and usually subsides within an hour [64]. In approximately 50% of cases, a late-phase response (LPR) develops that is also characterized by marked bronchoconstriction [64] (Fig. 2). In mice the primary physiological outcome parameter is usually AHR, which has been demonstrated using a multitude of allergens and sensitization techniques [65]; however, the acute responses to allergen inhalation are poorly defined in mice and in particular it is unclear as to whether mice, as a model, are capable of exhibiting a physiological late-phase constriction in the lung [65, 66] (Fig. 2).

One of the major criticisms of the most common mouse models is the lack of chronicity of the response to allergen exposure following sensitization [67]. If a mouse is sensitized systemically with an antigen and then challenged repeatedly via the airways, they have been shown to develop tolerance to the allergen, whereby the immunological response is suppressed [68]. Recently, a mouse model of asthma using repeated low-dose allergen inhalation has been developed that results in airway remodelling that is more typical of human asthma [34, 69] (Fig. 1). Similarly, models using sensitization and chronic exposure via the respiratory routes with HDM extract, an antigen that is clinically significant in humans, have shown that is possible to induce a pathology that is similar to human asthma with airway eosinophilia and long-term sensitivity to bronchoconstricting agents [70]. Such models represent a promising area of research and may negate previous criticisms regarding the appropriateness of mice as models of asthma.

Mice are currently and, in all likelihood, will remain the most popular model simply because of the technology available to manipulate their biology. They are an effective means of generating hypotheses that can then be tested in human asthmatics.

Rats

Rats are also popular as models of allergic airways disease. Like mice, they are relatively cheap, which allows large-scale studies on multiple outcomes to be conducted. Historically, rats were probably more popular than mice but have been overtaken in recent years due to the rapid expansion of genetic technologies associated with mice. From a logistical point of view, rats represent a significant advantage over mice due to their larger size. This makes it easier to measure the classic characteristics of allergic airways disease such as airway and systemic markers of inflammation due to an increase in the volume of serum and BAL fluid that can be obtained. This larger size and their increased stability under anaesthesia may also be seen as an advantage in terms of measuring physiological outcomes such as airway hyperresponsiveness and acute responses to allergen inhalation. There are a number of strains of rat available, the most popular being the Brown Norway (BN), with considerable variation in the response following sensitization and challenge between these strains [11, 71, 72]. Compared with mice, the range of

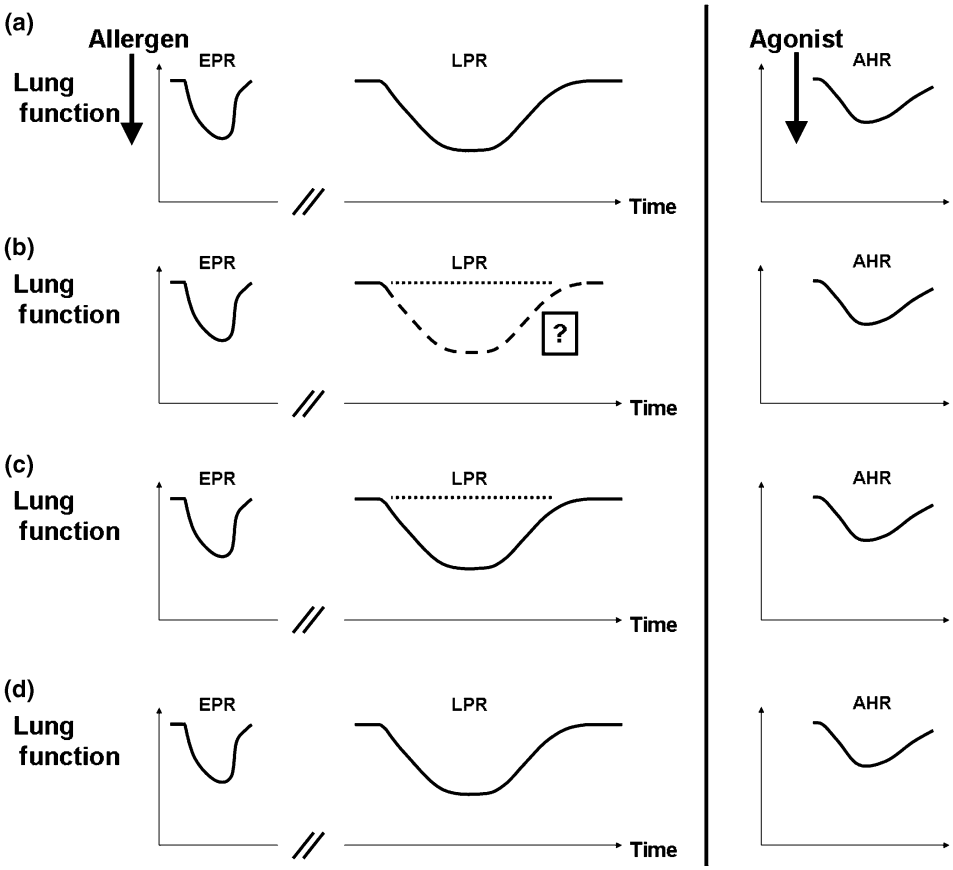

Fig. 2. Schematic representation of the potential physiological responses to inhaled allergens in terms of early-(EPR) and late-phase responses (LPR) and airway hyperresponsiveness (AHR) to non-specific bronchoconstricting agents. (a) is representative of the system that is being modelled, the human asthmatic, where there may be three components of physiological dysfunction in the lung: an EPR, an LPR and AHR. In contrast, in the mouse model (b), while there is evidence of a physiological EPR and AHR, evidence for an LPR is controversial. Other animal models such as rats, guinea-pigs and dogs (c) have been shown to develop an EPR, LPR and AHR (although the evidence for these, in particular the LPR, is highly dependent on the protocol used). (d) Sheep are currently the only animal model that have been shown to develop an EPR, LPR and AHR where there is a link between the acute allergic response and the development non-specific hyperresponsiveness.

reagents available for studies in rats is more limited but their availability has increased in recent years. Also, with the advent of the transgenic technology in rats [73] our understanding of and capacity to manipulate rat genetics will allow further exploration of the mechanisms of allergy in rat models of asthma.

Like mice, rats can be easily sensitized to a range of neo-antigens such as OVA [72, 74], HDM extracts [74] and *Ascaris* antigens [71]. Again, sensitization and challenge in rats typically results in a Th2 dominated response characterized by eosinophilia and antigen-specific IgE [76]. As mentioned, the level of responses in these markers of immunology and inflammation is highly dependent on the strain used [75]. BN rats are known to have a more pronounced IgE and inflammatory response to challenge following sensitization compared with other strains [72]. This provides an opportunity to dissect the mechanisms driving the asthmatic phenotype and, like the mouse, this variation, combined with knowledge of the genetics and immunology of the species, is one of the primary advantages of using inbred rats as a model for asthma.

Sensitized rats have been shown, using appropriate methods for measuring lung mechanics, to not only demonstrate increased responsiveness to non-specific bronchoconstricting agents [76] but also acute responses to allergen inhalation [77, 78]. The acute response in BN rats is characterized by both an EPR and an LPR that peaks approximately 6–8 h after challenge [77]. In humans, there is an established link between acute responses to allergen inhalation, in particular the LPR, and the development of non-specific AHR [79, 80]. Consequently, the ability to generate an EPR, LPR and AHR in rats (Fig. 2) represents a significant advantage over mice as the link between the immunological events that drive acute physiological responses to allergen inhalation and the development of non-specific AHR can be investigated. Rats are often used as a standard model for testing new drug

therapies; in particular, the efficacy and toxicity of new drugs are often tested in rats before proceeding with clinical trials [81].

As with mice, one major criticism of rat models of asthma is the tolerance that develops with increasing allergen challenges following sensitization, which essentially, prevents the development of a chronic allergic response and the associated changes in lung structure and function seen in asthmatics. While it would appear as a downfall of the rat and mouse models of asthma that it is difficult to implement a multiple challenge protocol that results in the chronic changes in the airways associated with asthma, some would consider the development of tolerance as a model of interest in understanding allergic disease. These models of tolerance in rats have highlighted the importance of a subset of T cells known as T regulatory cells (Treg) in suppressing allergic responses [82].

Rats, like mice, are easy to work with and can be easily sensitized and challenged in order to induce allergic responses in the airways. They are advantageous over mice in some ways but also share some of the deficiencies seen in mouse models of asthma. They have, however, been useful in understanding the mechanisms of asthma and have been beneficial in recent times in understanding the modulation of tolerance in allergy.

Guinea-pigs

Guinea-pigs represent one of the oldest animal models of allergic airway responses, with studies conducted on these animals over 100 years ago [11]. Guinea-pigs are limited in terms of mechanistic studies, particularly those involving genetics, due to the low number of inbred strains and lack of guinea-pig-specific reagents available. As with the rodent models of asthma mentioned above, guinea-pigs are readily sensitized to OVA [83, 84] and it is easy to elicit a response to challenge that is similar to an asthmatic phenotype that involves eosinophilia and increased airway responsiveness [85].

The guinea-pig is the most widely used model and test system for contact hypersensitivity to chemical irritants and proteins. Guinea-pigs are also often used as a 'screening' model for drugs that act through particular pathways that are seen to be relevant to human asthma and have been useful in the development of drugs such as corticosteroids and β_2 receptor-agonists [81]. The response of isolated guinea-pig airways to pharmacological agonists has been compared directly with humans to determine whether they are a good model for human airways [86]. It was found that there were similar responses between human and guinea-pig airways when exposed to methacholine, histamine and allergen following sensitization. There was, however, a difference in the response to leukotrienes, which suggested a difference in the mechanism of action between guinea-pigs and humans and serves to highlight the care that must be taken when making broad generalizations about the relative merits of one species as a model for human disease. Similar studies on precision-cut lung slices found more agreement between pharmacological responses of guinea-pig and human airways than that seen in rodents, with the exception of serotonin, which is a potent bronchoconstrictor in guinea-pigs but not humans [87]. Ressmeyer et al. [87] also found a discrepancy in the response of the guinea-pig through the leukotriene pathway with a comparable response to the direct agonist LTD_4 but a weak response to montelukast, which is known to be an effective antagonist of this pathway in humans [88]. Again, care must be exercised when using animals as a screening model for drugs as there is plenty of opportunity for false-positive and false-negative results. Having said that, it is also possible to obtain false-positive responses to therapies in the clinical setting whereby certain drugs are particularly effective in moderating aspects of asthma in humans, such as inflammation, but have little or no impact on the flow limitation associated with morbidity and hospitalization.

In mimicking the physiological abnormalities of human asthmatics, guinea-pigs may be seen as an ideal model as they develop a well-characterized EPR and LPR to allergen challenge following sensitization [89]. Again, most of the focus on early- and late-phase reactions by the airways in guinea-pigs has been related to responses to irritants rather than classical 'atopic asthmatic' reactions. An interesting feature of the guinea-pig acute response to chemical irritants is the extreme nature of the response, which is known to be lethal in some cases [11]. In order to manipulate this as a model for occupational asthma, guinea-pigs are often pre treated with antihistamines to moderate the response [11]. Guinea-pigs also develop post-mortem bronchconstriction, which is thought to be mediated by local release of substance P into the airways and requires pre-treatment with capsaicin or isoproterenol for experiments involving post-mortem manipulation of the airways [87]. The pre-treatments that are required in order to make the guinea-pig a workable model of allergic airway reactions need to be considered when extrapolating results to humans.

From an immunological point of view, IgG1 seems to play a critical role in allergic responses in guinea-pigs [81]. After repeated challenge guinea-pigs also become tolerant to the allergen [90]. Guinea-pigs are known to have a high baseline level of eosinophils which may confound the results of experiments conducted over a long period of time.

Guinea-pigs are useful as models of immediate hypersensitivity to irritants and have pharmacological

responses similar to humans, although care must be taken when applying this to all pathways in the human lung.

Larger animal models. All of the models of asthma mentioned so far are relatively widespread which is due, in the most part, to their availability and relatively low cost. There are a number of larger animal models of asthma that are used that have advantages over rodent and guinea-pig models of asthma due to the ease of measuring outcome parameters associated with their larger size. These models, in terms of the quantity of studies that have been conducted, are nowhere near as popular as the previous models but this is probably simply due to their cost and the lack of specific probes available for studying their allergic responses.

Dogs

Dogs have been used for some years as models of asthma. It has been suggested that dogs represent an ideal model of allergy as they have a natural pre-disposition to develop allergic responses to antigens that are clinically significant to atopic humans [33]. This allergy usually manifests itself in superficial reactions in the form of dermatitis or conjunctivitis rather than asthma like reactions in the airways [33]. It has also been shown that a certain level of eosinophilia develops in the airways in response to allergens but this inflammation does not result in an increase in responsiveness of the airways [91]. This discrepancy has been linked to the unique anatomy of the dog airway which is proportionally larger than most mammal species and may, therefore, be less prone to the development of airway constriction [91].

However, there are a number of dog models that display the hallmarks of atopic asthma including increased IgE [92], eosinophilia [93], acute physiological constriction in response to allergen inhalation [93] and AHR [92]. Dogs can be readily sensitized and challenged with *Ascaris suum* to elicit an allergic response in the airways [94]. A model using Basenji greyhounds has been developed but these animals have an increased baseline AHR that may not necessarily be associated with any clinical symptoms or pathology [36]. There is also a significant level of neutrophilia associated with the response that is far greater than that seen in atopic asthmatics [36].

More recent models have taken advantage of the predisposition of dogs to develop allergy by selectively breeding male and female dogs with high IgE titres. Approximately half of the offspring inherit high levels of IgE which has been likened to the inheritance of atopy in humans [92]. The offspring are sensitized to ragweed via a series of intraperitoneal and subcutaneous injections [92]. The sensitized dogs are then challenged with one to three bouts of aerosolized ragweed extract and the response measured. The airway challenge results in increased IgE, eosinophilia and airway responsiveness to both histamine and the allergen itself. Of particular note, compared with all the previously mentioned models, this is the first that shows prolonged AHR following challenge with an increase in responsiveness noted up to 5 months after the third challenge [92]. The main problem with this model is that it is extremely labour intensive and expensive but may prove particularly useful in identifying mechanisms of disease, in particular those relating to long-term changes in lung function.

Sheep

Like certain dogs, sheep are known to be naturally sensitized to *A. suum*. There is a natural variability within sheep that mount an allergic physiological response to inhaled allergen [95]. This variability within responders mirrors the pattern of variation seen in humans. The response to challenge in sheep is characterized by an influx of inflammatory cells into the airway which includes eosinophils and neutrophils [96]. These allergic responses also demonstrate a pattern of mediators that are similar to human asthmatics, in particular the leukotriene LTE_4 [97]. In terms of bronchoconstrictive responses, this model is able to demonstrate both immediate physiological responses to inhaled allergen, in the form of an EPR and LPR, and non-specific AHR. As mentioned previously there is variation between responders with some having single responses, an EPR, and 30–50% having dual responses, an EPR and LPR [95], which is a similar to the level of variation between human asthmatics [98]. Of note, is the fact that it has been demonstrated that only those sheep that develop a LPR have AHR 24 h after challenge [99] (Fig. 2), which again, is similar to asthmatics [79, 80]. In terms of drug efficacy, there are also striking similarities between this model and asthma with cromolyn and corticosteroids being effective therapies for allergic airway responses in the sheep [100]. However, despite the attractiveness of this model, it should also be pointed out that there are some discrepancies between the responses in this model and humans. One particular example is the early interest in platelet activating factors. Drugs that worked through this pathway and were shown to be effective in moderating lung allergic responses in sheep [101] were found to have poor efficacy in humans [102]. This again highlights the care that must be taken when comparing animal models with human diseases. As with dogs and other large animal models of asthma, the increased ease with which allergic responses can be measured is often outweighed by the cost and labour associated with working with such models. This is highlighted by the limited work on airway remodelling, a hallmark of chronic asthma, in this species which may be

related to a reluctance to sacrifice animals due to their cost [36].

Problems with animal models

Extrapolating to humans

The most common criticism or question that is directed at the use of animals as models of asthma, or any disease for that matter, is; how can the results obtained be extrapolated to humans? In the previous sections some species-specific differences between human asthma and induced asthma like responses in laboratory animals have been identified. The importance of this point, however, requires a more general discussion about the generic differences between human asthmatics and animal models of asthma.

As mentioned previously, with the exception of a couple of specific examples, the kinds of animals that are used in laboratory experiments do not spontaneously develop a condition that can be labelled as asthma. Consequently, in order to study asthma using animal models an artificial asthmatic like reaction has to be induced in the airways. There is a vast literature on animal models of asthma with a commensurate number of methods for inducing 'asthmatic' reactions in the airways. This variation in technique is a particularly important point as it can have a significant impact on the outcome and is often overlooked when comparing responses between studies. Despite this enormous variation, the general technique for inducing 'asthma' in laboratory animals involves sensitizing their immune system to a previously unseen antigen and subsequently challenging the airways with the same antigen in order to study the cascade of events or a distant outcome parameter of interest that occurs some time after the challenge. There are an enormous number of techniques for achieving this and it is not possible to provide a comprehensive list but a good starting point is the general OVA protocol that is the most common and is used primarily in the smaller species including mice [41], rats [72] and guinea-pigs [84]. This technique usually involves sensitizing the animal systemically via intraperitoneal injection with the antigen and an adjuvant, usually aluminium hydroxide (alum), to prime the immune system to respond in the desired way [103]. This injection may be done singularly or with booster injections and is followed after a predefined incubation period, usually 1–2 weeks, with an aerosol challenge with OVA. This technique results in an immunological response in the airways that has some of the inflammatory hallmarks of asthma. Samples are then taken some time after the challenge, usually 24–48 h post-challenge, depending on the time-point and process of interest. There are a number of issues with this technique, which are generic to all species of lab animal when this technique is applied. These relate to the design and biology of the models and will be discussed below.

Adjuvants

A typical senitization protocol involves injecting an antigen of interest in the presence of an adjuvant [103]. OVA is the most popular antigen because it is readily available and the environment, via diet, can be easily manipulated such that the animal's immune system has not been exposed to OVA before senitization. Adjuvants such as alum are known to promote the development of a Th2 phenotype by the immune system when it is exposed to an antigen [104]. A number of adjuvants other than alum are used in animal models of asthma including heat killed *Bordetella pertussis* [105], ricin [78] and adjuvant mixes that are known to promote a more Th1 biased response such as Freund's complete adjuvant [106]. The problem with the use of such adjuvants is it may alter the mechanisms of senitization to an allergen that are being modelled and the way in which the immune system has been primed may further remove the animal model from the human condition.

In an attempt to remove adjuvants as a variable from the equation, a number of models have been developed that do not require the use of adjuvants. These can be loosely categorized into conventional sensitization by exposing the immune system to the antigen vs. adoptive methods of sensitization whereby primed or altered cells are transferred to naïve recipients. There are number of examples of the first style of adjuvant free sensitization. Repeated intranasal sensitization of A/J mice has been shown to result in an asthmatic like response in the lungs [107]. This method is also thought to be more like the human condition in that sensitization occurs via the target organ of interest rather than by systemic priming of the immune system in general [107]. However, the utility of this model appears to be strain specific as both the BALB/c and C57BL/6 strains demonstrate a much milder response to this type of senitization and challenge but a more potent response to the classic intraperitoneal OVA senitization model than the A/J mouse [34]. Adoptive transfer models are also common in the mouse literature as a model of asthma. These models may involve transferring dendritic cells that have been pulsed with antigen in culture [108], primed wild-type T cells [109] or genetically altered T cells that innately recognize the antigen of interest into naïve recipients [110]. The recipient mice are then challenged via the airways with the corresponding allergen of interest. These techniques, while not as common as active senitization methods, have been found to demonstrate airway inflammation dominated by eosinophils [111] and AHR [109, 112].

Chronicity

One of the repeating themes in the discussion above of the relative merits of particular species was the issue of chronicity. Asthma, in general, is characterized by substantial remodelling of the airways which includes, but is not limited to, subepithelial fibrosis [113, 114], goblet cell metaplasia and hyperplasia [115], mucus hypersecretion [115, 116] and thickening of airway smooth muscle [117, 118]. This remodelling is thought to be a result of repeated exposure to allergen which causes repeated or continuing inflammatory events in the airways [119]. As a consequence, the airway wall structure is altered which has an impact on airflow and may be linked to the non-specific hyperresponsiveness that is typical of asthma [120]. The bulk of the models that have been mentioned so far lack these chronic markers of inflammation and long-term AHR [58]. Most of the models are in fact models of acute inflammatory events in the airway in the setting of a primed immune system. In particular, it is well established that mice, rats and guinea-pigs actively sensitized to an antigen will respond to inhalation of the antigen initially but if the challenge protocol is repeated the animals will usually become tolerant to the allergen [58, 82, 90].

This phenomenon has been of interest in studies of the development of tolerance as a pathway that may be mimicked to prevent the development of an allergic response [82]. However, if the intention is to understand the mechanisms of chronic asthma these models are clearly deficient. There have been attempts to develop models of asthma that show more chronic elements of the disease. Temelkovski et al. [69] used a mouse model with a systemic method of senitization followed by repeated low dose aerosols of OVA and were able to demonstrate some of the chronic changes seen in asthmatics. There are some models, including selected mouse models [70], already mentioned in previous section, that appear to have long-term AHR. One example is the dog model involving selectively bred high IgE titre producing individuals sensitized to ragweed [92]. Following three challenges with ragweed it was found that AHR can still be observed up to 5 months post-challenge. There is, however, no model in smaller laboratory animals that demonstrates both chronic remodelling and long-term AHR and the dog model of asthma has other disadvantages including the cost and labour associated with working with such a model. As yet, current models of asthma are limited by their lack of chronicity which must be taken into account when interpreting results of mechanistic and interventional studies before extrapolating the findings to humans.

Infants vs. adults

The progression of asthma as a disease is well known to be influenced by genetic factors, with certain individuals being predisposed to the development of an allergic phenotype [3]. These genetic factors combined with environmental factors will determine whether an individual will go on to develop asthma. While there are a subset of individuals that experience late or adult onset asthma [121], it is well recognized that early life events which result in immunological and structural alterations in the lung are critical in determining whether an individual goes on to develop asthma. These events include the *in utero* environment [122], viral infection [123], exposure to allergens [124] and other environmental factors such as smoking and pollution [122], pets [125] and bacterial by-products [126]. Having identified the importance of these early life factors, one of the primary problems with the animal models of asthma discussed so far becomes immediately apparent; these models use adult animals. Genetic pre-disposition based on the phenotype of the parents can be modelled using laboratory animals. As mentioned previously, there are dog and sheep models whereby the animal is pre-disposed to allergy to a specific allergen which are useful in terms of a model that more closely mimics the case of an atopic asthmatic where the importance of an inappropriate systemic response to a particular antigen is a well recognized risk factor for developing asthma [27]. Similarly, the genetic variation between strains of mouse may be used to understand the heritability of traits that are typical of the asthmatic phenotype such as AHR [127]. The *in utero* environment has also been modelled using animals. For example mouse models of asthma involving senitization during pregnancy and the subsequent study of the offspring have been conducted [128].

In terms of relative quantity, there are few animal models where numerous probes are available, such as the mouse, rat and guinea-pig that use a method that involves senitization of neonates or juveniles. This is particularly important given that it is known that there are changes in the immune system during development from early life to adulthood [129]. Consequently, sensitizing an adult immune system to an antigen, as in the case with most animal models, is likely to be vastly different to sensitizing a juvenile animal when the immune system is relatively naïve and immature. Without an established model of sensitization and challenge using neonatal animals it will not be possible to directly examine the influence of factors such as viral infection and environmental exposure which are known to be critical in the development of asthma in humans [122].

Anatomical

Most of the previous discussion has focussed on design and immunological issues associated with animal models of asthma. Another factor that needs to be taken into consideration when extrapolating data to humans is the

anatomical differences between species of mammal. The major clinical symptom of asthma, and the one that directly results in hospitalization and morbidity, is difficulty in breathing. This difficulty in breathing is largely a result of airflow limitation. When developing models of asthma using animals, the ultimate aim should be to mimic the clinical outcome so that the inflammatory mechanisms leading to this outcome can be understood. Before designing a model of airflow limitation the factors that may influence this outcome in asthmatics need to be identified. Airflow limitation in a tube, the simplest model of the airway, may result from constriction, collapse or blockage of the tube. Blockage, constriction or collapse in the asthmatic airway may be due to a number of factors such as thickening of the airway wall [119, 130], increased responsiveness of the airway smooth muscle [131], inflammatory exudates [132] and alterations in the parenchymal-airway interaction [131]. There are some issues with animal models that relate specifically to these factors that need to be considered when interpreting physiological data. One obvious difference between humans and animal models of asthma is the fact that all of the animal models discussed so far are quadrupeds. This may seem like an inconsequential difference, however, this difference in posture may have a significant impact on the forces acting on the lung due to the effects of gravity and the chest wall [133, 134] which may influence the way the lung responds to flow limitation.

The branching pattern and morphology of the airway also varies considerably between mammal species [135–137] (Fig. 3). The branching pattern may have a significant impact on aerosol deposition [138] which would influence both the delivery of an allergen to the airway and the distribution of bronchoconstricting agents to various parts of the lung during tests of AHR. Variations in lung morphology between species are also seen in the size of the airways with dogs having proportionally large conducting airways [91]. Similarly, it has been shown that there is an inverse relationship between body size, in different species of rodent, and relative airway calibre [139]. Structurally, mice also have a relatively low proportion of airway smooth muscle in their airways [11] which may limit their utility as a model for altered smooth muscle function which results in increased constriction in the airway. The relative proportion of cell types in the airway varies considerably between species [137] with mice seeming to have a relatively high number of clara cells which are thought undergo metaplasia following allergen challenge to form unusually large goblet cells in the airway [57]. It is also important to note that the distribution and class of receptors along the airways that respond to pharmacological agents differs between mammals [87].

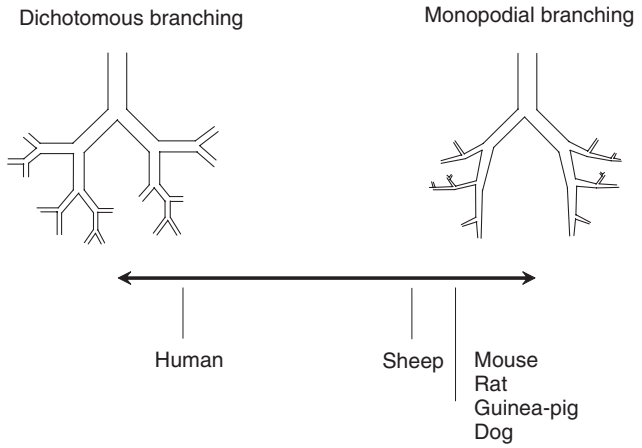

Fig. 3. Schematic representation of different lung branching patterns ranging from dichotomous, where the daughter branches are equal in diameter, to monopodial where one of the daughter branches is much larger (major) than the other (minor) (adapted from [135–137]). The human branching pattern is the most symmetric of all the mammals studied and is almost perfectly dichotomous. All of the species of animal model described in this review have a strongly monopodial branching pattern, although there is evidence, in sheep at least [154], that the smaller airways may have a dichotomous pattern.

Size

The size of the animals described so far is also an issue in terms of measuring outcomes of interest in models of asthma. This issue is particularly important in terms of measuring lung function. The measurement of lung function in smaller animals, and in particular mice, represents a significant technical challenge. Aside from the technical issues associated with measuring parameters such as flow due to the problem of dead space when measuring such small volumes [140], there are a number of technique specific issues that need to be recognized. The techniques currently available in mice, for example, include non-invasive *in vivo* [141], invasive *in vivo* [142] and *in vitro* techniques [143]. If these techniques are arranged in this fashion on a spectrum from the least invasive to the most invasive a generic problem with measuring lung function in small animals becomes apparent. The most accurate measurements require the most invasive techniques and, conversely, less invasive techniques provide data that may be of questionable quality. This problem lead Bates and Irvin [144] to propose the 'phenotyping uncertainty principle' whereby the most precise measurements of lung function require techniques that are far removed from natural breathing conditions but, on the otherhand, by using a technique that attempts to monitor the animal under natural conditions there is a significant sacrifice in precision which may render the data unusable. Bearing this problem in mind, and using the mouse as an example, there are a number of techniques with specific advantages and disadvantages that should be mentioned when considering experiments using animal models of asthma.

In recent years, the most popular technique, by far, in terms of prevalence in the literature, has been the use of non-invasive barometric plethysmography in conscious mice and measuring a parameter known as enhanced pause (Penh). Penh is calculated from inspiratory and expiratory time and peak inspiratory and expiratory pressure which are derived from fluctuations in box pressure [141]. It was claimed that Penh could be used as a proxy for airway resistance because of the apparent correlation between this parameter and changes in respiratory patterns due to airway constriction [141]. However, there are a number of problems which include, but are not limited to; (1) Penh is highly dependent on breathing frequency [145] (2) mice are obligate nose breathers so Penh will be influenced by upper airway inflammation [65] (3) changes in box pressure will be due to both compression and rarefaction of the air in the box and changes in humidity with only the former being related to lung function [146]. These issues have lead to widespread criticism of this technique [147].

In terms of more invasive techniques whereby anaesthesia, tracheostomy or intubation and mechanical ventilation are required, the simplest measurement is probably the airway-pressure time index (APTI). The APTI is calculated as the area under the airway-pressure curve at a fixed tidal volume following application of a bronchoconstricting agent [148]. This gives a global measure of the effective impedance of the respiratory system but is not able to partition changes in impedances into airway and parenchymal components. The relative contribution of the resistive and elastic compartment of the lung to overall impedance, which is the pressure required to generate a particular flow, can be calculated using a single compartment model based on the equation of motion;

$$P = R\dot{V} + V/C_{\mathrm{dyn}}$$

where P is the transpulmonary pressure, R is the resistance, $\dot{V}$is the airflow, V is the volume and C_{dyn} is the dynamic compliance [149]. The values obtained from this equation are dependent on the frequency of the signal used to generate the flow so it is possible to generate further information if a signal is used that contains multiple frequency components. The forced oscillation technique uses a multiple frequency sinusoidal signal to generate an impedance spectrum (Zrs). This signal can be applied via the airways, to calculate the input impedance using a piston to generate the oscillations [150] or a loudspeaker where the impedance is calculated as the load impedance on wavetube [151], or via the body surface to calculate the transfer impedance [152]. Having generated the Zrs it is then possible to fit a model to the data obtained to allow estimation of airway and parenchymal parameters [153] (Fig. 4).

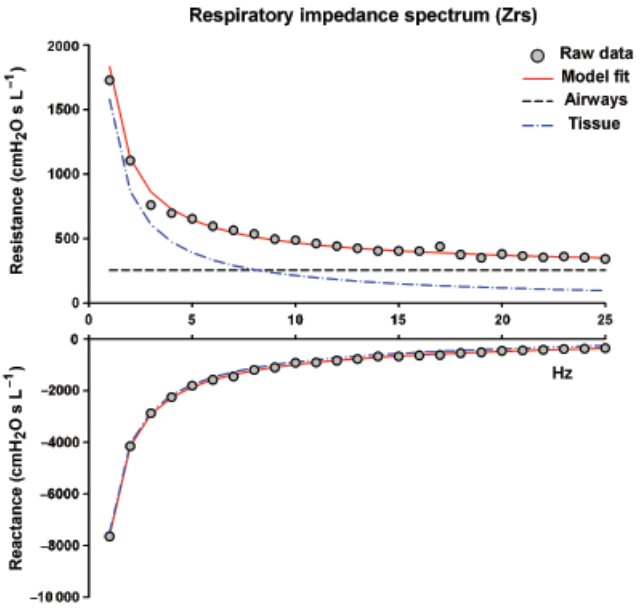

Fig. 4. Representative respiratory impedance spectrum (Zrs) from an anaesthetized, tracheostomized mouse showing collected data (circles), from an oscillatory signal containing frequency components from 1 to 25 Hz, the model fit based on the constant-phase model [153], which is a four-parameter model and allows calculation of airway resitance (R_{aw}) and inertance (I_{aw}) and the parenchymal indices tissue damping (G) and tissue elastance (H) (red line). The dashed lines represent the relative contribution of the airways (black) and tissues (blue) to the spectrum.

In recent years there have been significant advances in the techniques available for measuring lung function in animals as small as mice. This has allowed more detailed assessment of animal models of allergic airways disease. However, care must be taken to recognize the limitations of the technique that is being used so that appropriate interpretations of results can be made.

Conclusions

Despite the concerted efforts of scientists and clinicians around the world, the pathobiology of asthma is still relatively poorly understood. The recognition that asthma is a heterogeneous disease involving a number of pathways, and the ethical issues associated with the required studies in humans, has required the development of tools to assist in mechanistic studies of asthma. The tool that allows the most effective investigation of disease mechanisms and progression involving an intact respiratory and immune system is the animal model of asthma. There are a wide array of models available for study both within and between species. Each model has its own limitations and advantages which must be recognized when designing studies and interpreting the results that are obtained. Issues that need to be considered include immune and anatomical differences between the animal and humans. The absence of asthma as a natural disease in the animals

that are used requires artificial sensitization and challenge with most models only being suitable for the study of acute inflammatory events. At present, a majority of models are limited by the use of adjuvants and adult animals in their design. Future studies should recognize this and pursue adjuvant free models of disease that use sensitization during the first stages of life as it would occur in human asthmatics.

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