

Immunotherapy mouse models for allergic asthma: what are they good for?

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7/27/2017



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Abstract

Allergic asthma is a chronic inflammatory disease of the respiratory system, characterized by airway inflammation, reversible airway obstruction, and hyper-responsiveness of the airways. Current treatment is mainly focused on treating symptoms. Immunotherapy is the only current treatment that is directed at treating the underlying mechanisms of allergic asthma instead of the symptoms.

The heterogeneity and complexity of allergic asthma makes the disease difficult to study. To continue studying the mechanisms of allergic asthma animal models are used, mainly mouse models. However, the translation of mouse studies to humans has been shown to be difficult. There have been many promising pre-clinical results, but there has been a lack of new drugs on the market. Therefore, the question rises, what the clinical relevance of immunotherapy mouse models for allergic asthma is.

There is not one single protocol which is followed regarding immunotherapy mouse models. There are many variations in the mouse models currently used. There are differences in mouse strain, used allergens, the use of adjuvants, the route of administration of the allergens, and the duration of sensitization and challenge. All these differences in protocol can influence the development of asthmatic symptoms in these models making it hard to compare these studies. Moreover, results have shown that current mouse models severely lack translational efficacy. These results show that the relevance of past years of allergic asthma research regarding mouse models is questionable.

Unfortunately, there has not been a new mouse model discovered with a better translational efficacy. Different animal models all have their own limitations. With new advancements in technology coming, there might be new models available in the future. The current mouse models are severely lacking in terms of translation efficacy but even though they lack translation efficacy they are still the best model to do the vast majority of early research. Extrapolating results from these studies to the human disease should be done with great caution.

Introduction

Approximately 300 million people are suffering from allergic asthma all over the world¹. Allergic asthma is a chronic inflammatory disease of the respiratory system and is characterized by airway inflammation, reversible airway obstruction, and hyper-responsiveness of the airways². In most cases, asthma begins to develop in childhood after sensitization of different kinds of allergens for example, House Dust Mites (HDM) or Grass Pollen (GP)³.

Pattern recognition receptors (PPRs) on the epithelial cells of the airways will detect inhaled allergens. After detection, these cells will activate the immune system by releasing different cytokines, namely: interleukin (IL)-1, IL-6, IL-8, IL-25, IL-33, granulocyte-macrophage-colony stimulating factor (GM-CSF), Interferon- α , and thymic stromal lymphopoietin^{4,5}. These mediators promote inflammation, through dendritic cells (DCs) and through type 2 innate lymphoid cells (ILC2s)⁵. DCs located just below the epithelial layer of the airways take up the allergen and migrate to draining lymph nodes to present the allergen to naïve T cells, which in turn differentiate into T helper (Th) 2 cells⁵. These Th2 cells release IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF which are responsible for B cell IgE production, eosinophil activation, hyper-responsiveness of the airways, and bronchial airway thickening due to an increase in smooth muscle and matrix proteins^{4,6}. This cascade of reaction is schematically and simplified shown in figure 1.

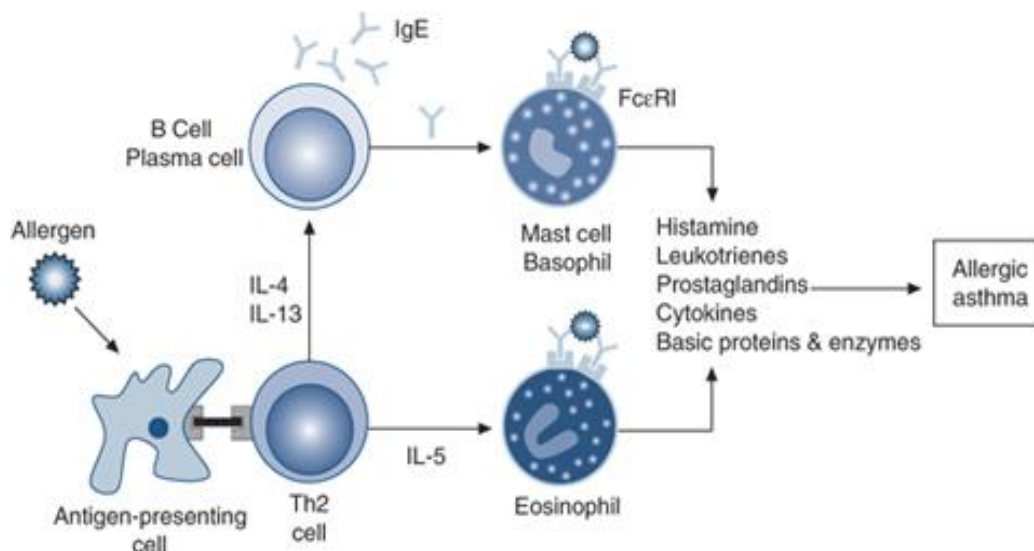


Figure 1. Cellular pathway from uptake of the antigen by DCs to allergic asthma ⁶

Symptoms of asthma consist of coughing, difficulty with breathing, chest tightness, shortness of breath, and wheezing⁷. Currently, most treatment is focused on treating the symptoms using corticosteroids. Another possible treatment is Immunotherapy; this is currently the only therapy that is aimed at treating the underlying condition of allergic asthma instead of the symptoms. In immunotherapy the patient is exposed to increasing concentrations of the allergen to desensitize the immune response. Immunotherapy can alter the responses of T cells in various ways. A switch in balance from Th2 cell pattern to Th1 cell pattern, induction of regulatory T cells, or deletion of pathogenic allergen-reactive T cells^{8,9}.

The complexity and heterogeneity of allergic asthma makes not only the management of the symptoms challenging but also makes the investigation of the underlying causes of the disease complex¹⁰. To study the mechanisms involved in allergic asthma, mouse models are used. These mouse models are also used to study specific interventions which cannot be tested in humans. However, there are many variations of this mouse-model making it difficult to compare different studies.

Many different pathological mechanisms have been unraveled with the use of mouse models. There also have been many promising preclinical studies with potential new targets using mouse models¹¹. Even with all these unraveled pathways and a large amount of promising preclinical studies few new drugs for allergic asthma have made it to the market¹¹. With these results and the knowledge that mice are fundamentally very different to humans the question arises, can the results from mouse model studies be extrapolated to humans?

Therefore, it is important to review the different mouse models available and what the advantages and the drawbacks of the different models are and if these models are clinically relevant. In the coming sections the different models will be discussed, an assessment of their clinical relevance will be made, and a suggestion for the most suitable immunotherapy mouse model will be made.

Different immunotherapy mouse models

Basis of immunotherapy mouse models

There are different ways of inducing tolerance using immunotherapy¹². The first method of inducing tolerance is by applying immunotherapy before sensitization and subsequent allergen challenges (fig. 2a)¹². Another possibility is applying immunotherapy after sensitization but before challenge, this method can be seen as a preventive therapy that could be used for people who have an IgE sensitization and are prone to develop allergic asthma but have not shown symptoms yet (fig. 2b)^{12,13}. The final way of inducing tolerance is by applying immunotherapy after sensitization and challenge, this method is comparable to a therapy for patients who already have developed allergic asthma (fig. 2c)¹².

In the method showed in figure 2b mice are injected with a specific allergen to induce sensitization of the immune system. Allergen sensitization is performed by either peritoneal, dermal, or subcutaneous routes¹⁴. Following this, mice will receive immunotherapy which can be performed either by sublingual administration (SLIT) or subcutaneous administration (SCIT). The immunotherapy consists of increasing concentrations of the allergen with or without adjuvant, but this will be further explained in the coming section. This increase will desensitize the immune system and should elicit

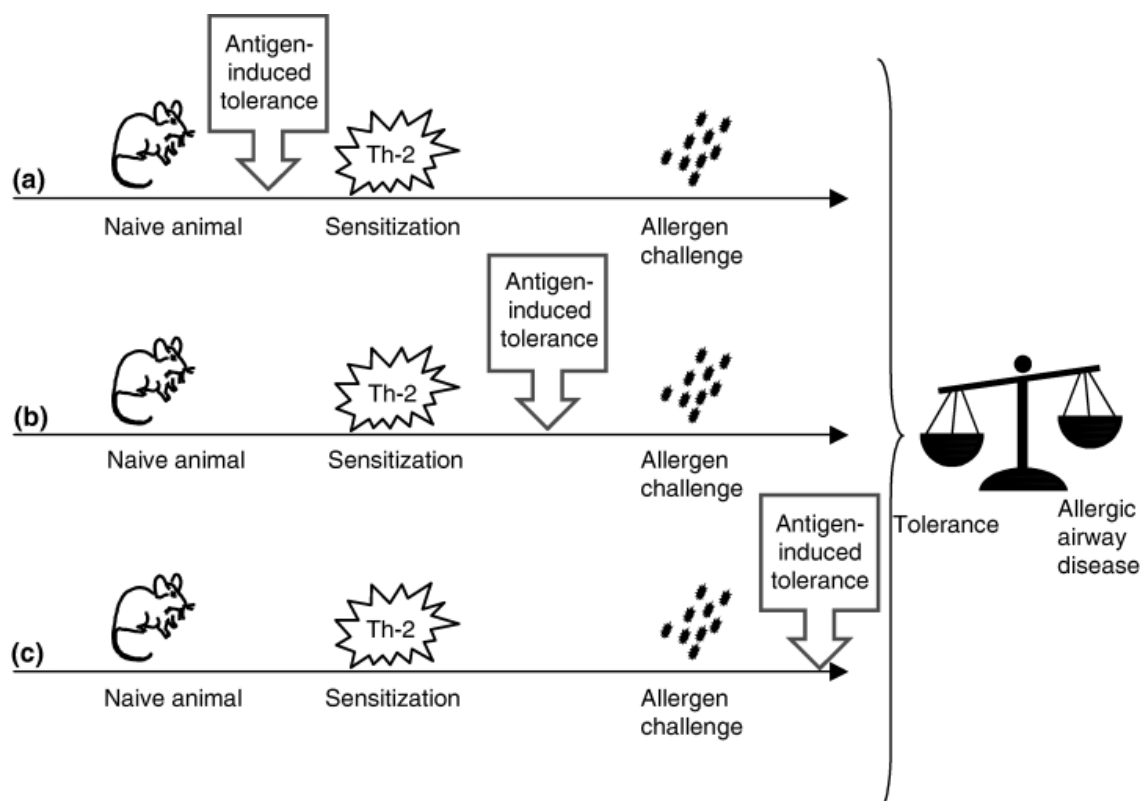


Figure 2. Different methods of inducing tolerance using immunotherapy in mouse models. a) Immunotherapy before sensitization. b) Immunotherapy after sensitization. c) Immunotherapy after challenge¹².

an immune regulatory response⁸. After immunotherapy the mice will be challenged, this is performed most commonly via aerosol allergens to examine how these mice respond to inhalation of the allergen. After successful immunotherapy the immune system should switch to a more regulatory response.

Differences in currently used models and their pros and cons

So far, there has not been a specific protocol which everyone follows for allergic asthma immunotherapy mouse models. Furthermore, there are a tremendous amount of different mouse models that are alike but not identical to each other. For example, there can be differences in mouse strain; the use of allergen; ways of administration of sensitization phase, immunotherapy phase, and allergen challenge phase; the use of an adjuvant; and in the duration of the different phases. In the coming section these differences will be shown and their advantages or disadvantages will be further explained.

Mouse strains

Already in the late 1990s different studies have shown that different mouse strains have different immune effects after immunization of a Th2-stimulant like aluminum hydroxide¹⁵⁻¹⁸. Currently, there are three different mouse strains commonly used in allergic asthma immune therapy models of mice, BALB/cJ mice, C57BL/6J mice, and FVB/NJ mice. Zhu et al. investigated the differences between the specific differences in immune responses to an Ovalbumin antigen within these three strains¹⁹. They showed that the three strains had significant differences in primary parameters like AHR response, Th2 cytokine output, and IgE antibody levels.

A large obstacle for human immunotherapy for allergic asthma is the risk of anaphylaxis. The risk of anaphylaxis is linked among others to IgE and IgG1 in humans²⁰. There are cases of IgE independent anaphylaxis, but for current models IgE and IgG1 levels in mice might be a good indicator for anaphylaxis risk of the mouse strain²¹. Among the previously mentioned three strains BALB/cJ mice presented the best separation of reactivity from controls as shown in figure 3, unfortunately Zhu et al. did not include the IgG1 figure in their report¹⁹.

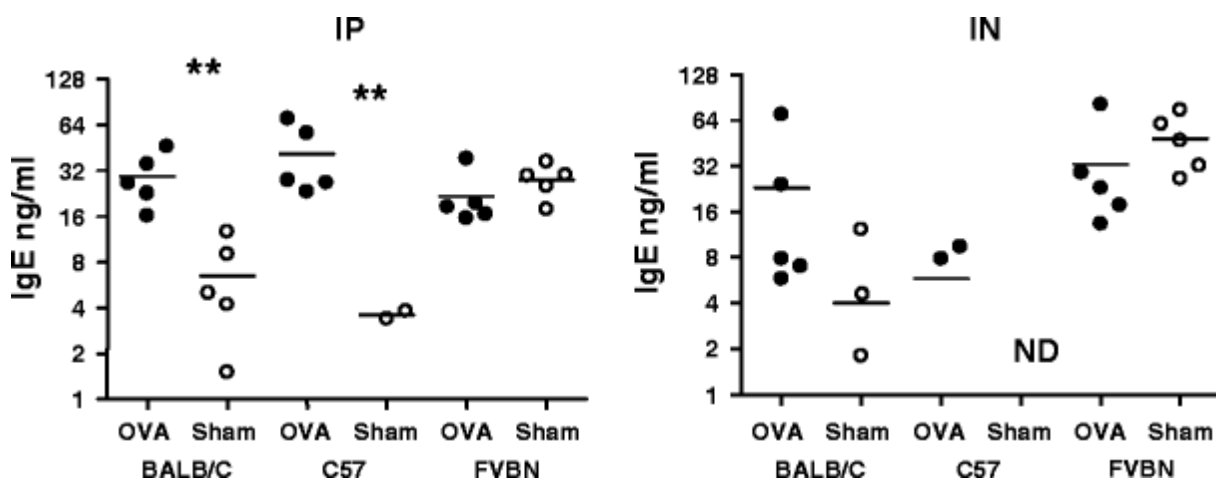


Figure 3. OVA-specific serum IgE levels from three strains of mice either saline- or OVA-sensitized and challenged with OVA¹⁹.

They concluded that these three mouse strains can all be manipulated to produce allergic lung disease and asthma, but BALB/cJ mice are the most reliable strain for most significantly expressing the markers for these diseases¹⁹.

Allergens

A lot of different allergens are widely used, for example OVA, House Dust Mites (HDM), and Grass pollen (GP). Commonly, models that use HDM or GP extracts use crude allergen extracts. However, because of many side-effects (adverse allergic reactions and even anaphylaxis) of these crude extracts there has recently been a shift to using specific allergens within the extract²². There have also been studies conducted on the use of peptides within these specific allergens to further improve immunotherapy²²⁻²⁴.

The large amount of different allergens used, be it whole extracts, specific allergens, or allergen-derived peptides make it hard to compare these studies because even though all these allergens are capable of inducing certain characteristics of allergic asthma in mice, they do have different effects.

OVA, often with an adjuvant like aluminum hydroxide, was classically the allergen of choice for mouse models of allergic asthma²⁵. The clinical relevance of these OVA-mouse models have recently been questioned¹¹. While asthma is a chronic disorder, OVA mouse models are acute models where the asthmatic symptoms resolve within weeks after final challenging¹¹. It is difficult to establish chronic OVA models since studies have showed that after repeated OVA exposure, mice gain tolerance^{14,26}. Also, OVA is not associated with human allergic asthma. All these findings diminish the clinical relevance of OVA mouse models.

Because of the above reasons antigens with a greater clinical relevance are also being used, like HDM and GP. Besides having a higher clinical relevance, HDM models do have a number of issues, studies have shown that in contrast to human asthma the mouse models using HDM as an allergen have fewer or even no increase in mast cells and levels of serum IgE²⁷⁻²⁹. Also, clinical immunotherapy with HDM or GP extracts showed to have a risk of many adverse side effects, including anaphylaxis³⁰. This is also the case in humans making these models even more clinically relevant. Some of these side effects can be lessened or in the case of anaphylaxis completely circumvented by the use of peptide immunotherapy instead of whole allergen immunotherapy^{23,31}.

Adjuvant

Another difference between models is the use of an adjuvant. An adjuvant is a substance that enhances the immune response to an antigen³². Adjuvants can not only enhance the immune response but can guide the response to produce the most desirable form immune response³³. These adjuvants can be either immunopotentiators but they can also be vectors. For example, an adjuvant is required for the use of most OVA-mouse models because OVA alone is often not antigenic. Broadly used adjuvants are Aluminum hydroxide and calcium phosphate³².

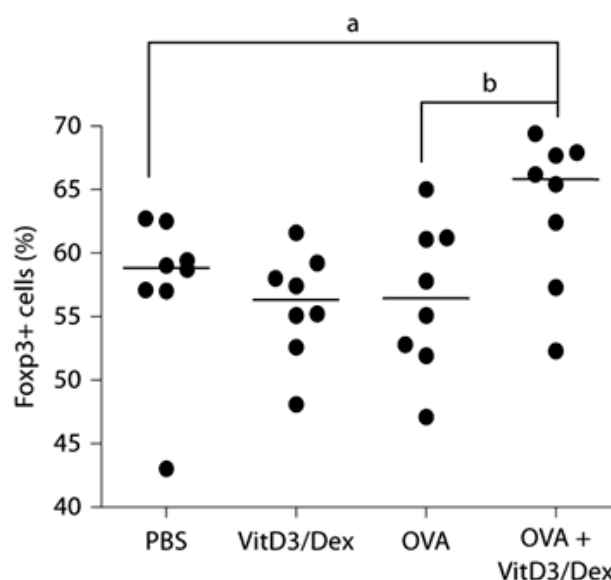


Figure 4. Foxp3+ cells after sublingual treatment with PBS, VitD3/Dex, OVA, or OVA + VitD3/Dex³⁴.

Many studies have shown the efficacy of adjuvants in immunotherapy for allergic asthma, for example Van Overtvelt et al. showed that a combination of 1,25-dihydroxyvitamin D3 and dexamethasone increases the efficacy of grass pollen immunotherapy in a BALB/c model³⁴. After immunotherapy in combination with the adjuvant there was a significant increase in Foxp3+ cells, which is a marker for regulatory T cells as shown in figure 4. This indicates a more immunoregulatory response compared to OVA immunotherapy without the adjuvant³⁴.

Adjuvants are not only restricted to mouse models, recently an increasing amount of research is being done regarding the use of adjuvants in a clinical setting. For example Sublingual immunotherapy (SLIT) combined with monophosphoryl lipid A (MPL) has shown to reduce reactivity to a subsequent allergen challenge in grass pollen allergic human patients³⁵. Figure 5 shows the effect of immunotherapy in combination with MPL in humans, a negative nasal challenge test (NCT) means a reduction in asthmatic symptoms³⁵. After the 8 weeks of immunotherapy the combination of the allergen and higher concentration of the adjuvant decreased the symptoms experienced by asthmatic patients.

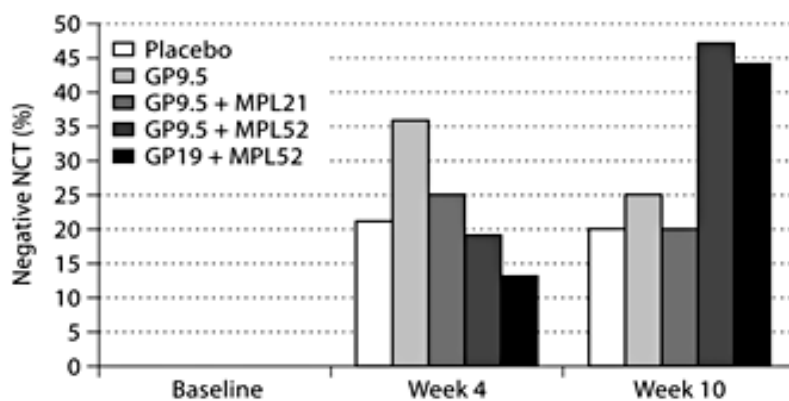


Figure 5. Percentage of negative NCT after 8 week immunotherapy with Placebo, Grass Pollen, and different concentrations of Grass Pollen in combination with MPL³⁵.

Besides immunopotentiators like MPL, more studies are also being conducted regarding the use of adjuvant vectors, but vectors have currently not been tested in humans³².

Even though adjuvants are also being used in immunotherapy, they are not part of the mechanism of how humans initially develop asthma. Therefore, the main issue with the use of adjuvants is that it might change certain mechanisms in a mouse model that even further distance the animal models from human conditions. Because of this, more questions regarding the translational efficacy of these OVA-models arise.

Administration

There are also many different ways in which these allergens (and adjuvants) are administered. Administration of the allergen in the sensitization phase is mostly done via either peritoneal, dermal, subcutaneous, intranasal, or aerosol routes^{14,36}. Each of these routes has differences in the outcome of developing allergic asthma symptoms in mice. Shown in figure 6 is an example of the difference in airway inflammation after intraperitoneal, subcutaneous, or aerosol sensitization³⁷. Increased levels of Eosinophils, IL-5, and Exotoxin suggest an increased airway inflammation.

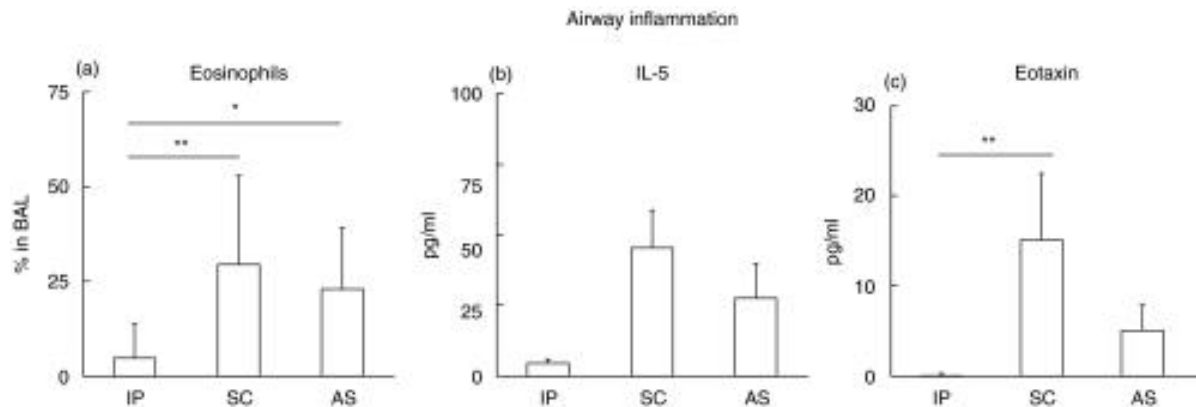


Figure 6. Assessment of allergic airway inflammation after intraperitoneal, subcutaneous, or aerosol sensitization with recombinant Bet v 1³⁷.

The subsequent immunotherapy can also be administered via different ways. The most common and clinically relevant are Sublingual Immunotherapy (SLIT) and Subcutaneous Immunotherapy (SCIT). Finally, Challenging is mostly done via the airways, intranasal, or by inhalation¹⁴. All these different ways of administration can have different effects on the immune response and on the disease pathology making comparison of different studies more difficult.

Both SCIT and SLIT are being performed in the clinic currently and both have their advantages and drawbacks. SCIT has been seen to be more effective than SLIT in the control of symptoms and in the reduction of antiallergic medication use³⁸. On the other hand, SCIT requires a long duration of doctor visits and injections while SLIT only requires the use of tablets and does not consist of multiple doctor visits, saving costs and being more easily and comfortably administered. But, SLIT does require up to 100 fold the dose compared to SCIT^{32,39}.

Returning to mouse models, SCIT is actually easier to administer compared to SLIT and since SCIT has better results in terms of symptom reduction, SCIT is the preferred choice in most mouse model studies. The downside here is that many mouse studies are using SCIT while SLIT is slowly becoming the preferred clinical and economical choice for immunotherapy³⁹.

Duration of sensitization and challenge

There are two major choices in the duration of sensitization and challenge: acute and chronic. Acute sensitization usually require multiple intraperitoneal administration of the allergen and often also need an adjuvant (although previously there are adjuvant free protocols described⁴⁰). After sensitization (and subsequent immunotherapy) the mouse will be challenged with the allergen for several days⁴¹. This is a relative short period of sensitization and challenge. The acute mouse models show many key features of allergic asthma in humans, they show elevated IgE levels, airway inflammation, and AHR⁴¹. However, even though there is airway inflammation present in these models, the pattern and distribution of this inflammation differs from that in humans for example, remodeling and matrix deposition was not present in the lower airways^{41,42}. Also, many of the present key features of allergic asthma resolve after a few weeks⁴².

Because allergic asthma is a chronic inflammatory disease resulting from continued or intermittent allergen exposure researchers started to use a more chronic mouse model. These chronic models consist of repeated intranasal allergen challenge, over periods of up to 12 weeks⁴¹. These models also show many key features of allergic asthma and in these models asthmatic characteristics like airway remodeling have been shown to persist, even after allergen challenging has stopped^{27,42}. The chronic models do have some downsides. For example, in contrast to humans inflammation is not restricted to the conducting airways⁴³, there is no increases in airway smooth muscle, and there are almost no mast cells present^{42,44}.

Future mouse models

The most commonly used immunotherapy model is largely dependent on what the researchers are trying to investigate, but the OVA mouse model has been a common choice for most studies. The OVA mouse model is cheap, convenient, easily reproducible, and mice have a short reproductive cycle. This model does not develop asthma as we see it in humans, but develops certain cellular and pathophysiological characteristics which are similar as seen in human asthma¹¹.

Unfortunately, there is no single mouse model yet to provide a suitable model of allergic asthma as it is seen in humans. And recently there have been no new models discovered to be a better model for human asthma. Asthma is not a simple disease, it has many features and it would not be logical to assume that a single animal model would be a suitable model of allergic asthma in humans. There are already a large amount of different mouse models suitable to investigate certain characteristics of asthma. The next step for future research with mouse models is to optimize the current mouse models and their protocols. Different mouse models could be used to investigate different effects, combining these different mouse models and combining the results is the logical next step. There is no single best option for a go to mouse model, but for clinical relevance it would be better to use a model with a clinical relevant allergen, such as HDM or grass pollen with an administration protocol that is most relevant to human clinical setting. For basic mechanism studies it would be better to use an OVA mouse model, since OVA is more easily reproducible than for example HDM or grass pollen.

To study more specific pathways and possible new therapeutic pathways there are other options besides mouse models. There are also *ex vivo* and *in vitro* models available. For example, excised bronchial segments could be used to study the airway smooth muscle. Another example of an *ex vivo* model are thin cut lung slices. Currently, this model is only used with lung slices from animals and not those from asthmatic patients. Human asthmatic lung tissue is characterized by thick mucus, which make it difficult to fill it with agarose, which is needed for this model⁴⁵. Besides the technical problems of using human tissue, there are also logistical problems. Human asthma patients generally do not undergo lung resections making lung tissue from human asthma patients scarcely available.

Translation of mouse models

Asthma is a complex and heterogeneous disease that has many different pathologies and characteristics. According to Mullane et al. there are several characteristics that an ideal animal model of asthma should include, these are: Similar genetic basis to the human, similar anatomy and physiology to the human, similar pathological response and underlying mechanisms of the human disease, employs similar endpoints as used in a clinical trial, responsive to drugs with known clinical efficacy, predictive of clinical efficacy¹¹.

Mullane et al. have written an extensive review describing above points step by step in regards to the OVA mouse model. Briefly summed up, the currently identified genes that contribute to asthma heritability in humans barely had any overlap in the corresponding genome-wide association study (GWAS) of the mouse model of asthma⁴⁶. There are many differences in the anatomy and physiology of the lungs in mice compared to humans¹¹. Regarding the pathological response and underlying mechanisms, some key features of the human disease also present themselves in the OVA-mouse model but many other key features do not present in the mouse model at all.¹¹ Current endpoints used in a clinical trials are not provided by most of the animal models¹¹. And finally, drugs with known clinical efficacy have shown varying results and new possible drugs have shown to have a poor translational success thus far¹¹.

The use of a more clinical relevant allergen, such as HDM or grass pollen, will result in a higher translational efficacy. But unfortunately these models will still have the same genetic, anatomic, and physiologic limitations.

Besides the OVA-mouse model, other mouse models of asthma can overcome some of the problems of the OVA-mouse model. But currently, different studies have shown that every single mouse model still lacks clinical translational efficacy. Of 39 anti-asthmatic drugs that have reached clinical trials (up until 2011) only 4 drugs have been shown to at least have limited efficiency while over 30 drugs have been discontinued⁴. An important note is that different drugs are effective for different asthmatic phenotypes and drugs deemed ineffective in one study might be effective for asthmatic patients with a different phenotype. For example, anti-IL-5 has been qualified as an ineffective drug for asthma patients but later studies showed that anti-IL-5 is actually an effective therapy for asthmatic patients with a more extreme type of asthma with an "eosinophilic phenotype"⁴⁷. This is data of all the drug trials that have been reported, since early stage (phase 2) negative clinical studies often go unreported there are possibly more clinical trials with negative results⁴⁸. Not all these drugs originate from mouse model studies, but these results do show the suboptimal clinical success rate of promising new drugs.

Discussion

Currently no mouse model that is currently used mimics allergic asthma as it is seen in humans, they only induce a pathological model with few asthmatic characteristics also present in the human disease.

There is no doubt that without mouse models we would have been even further away from helping the 300 million people suffering from allergic asthma and in the last 30 years of asthma research using mouse models there have been an immense amount of new information and insights uncovered regarding the understanding of different asthmatic characteristics, for example airway inflammation, the identification of key cells, cytokines, and pathways⁴⁹. Because of research with the use of these mouse models a vast amount of promising preclinical new drugs have been found. But unfortunately in the last 30 years few new treatments have been brought to the market or even in final stages of clinical trials and the few novel treatments that are emerging are unlikely to be more effective than currently used combination inhalers^{4,50}.

This suggests that the past years of research has been valuable in regard to gaining new insights but might have been a waste of time and money in regard to new drug development. Maybe now it is time to step away from the continuous stream of 'new and innovating' mouse studies and to look at more suitable, perhaps newer, better, and more promising models to study allergic asthma.

Ideally, asthmatic patients would be used to further expand our knowledge of allergic asthma. But asthma is a heterogeneous disease and one asthmatic patient would still not be the perfect model for another patient. Besides, due to ethical and logistical limitations this is not an option. Perhaps there are different animal models than mice available. Previously, studies have already been done on rats, guinea pigs, sheep, cats, and horses. Models of asthma in rats and guinea pigs also have their genetical and anatomical limitations. Non-rodent animals like sheep, cats, and horses might be a better alternative. Anatomical these animals might be a more suitable model but there are still many limitations, they are still genetically relatively different from humans, housing of these animals is expensive, and there would be ethical concerns using these animals as experimental models.

A more suitable animal model could possibly be non-human primates, such as rhesus monkeys. The vast differences between humans and mice results in a poor translational efficacy, but rhesus monkeys are genetically, anatomically, and physiologically more similar to humans making them a better model. In the past, rhesus monkeys have

already been used and do have an important part in preclinical drug screening in asthma studies. Because of all the failed attempts of discovering new drugs using mouse models, a consideration could be made to use rhesus monkeys in an earlier stage of drug discovery. This should result in a higher translational efficacy but this has major ethical concerns and is therefore not an option.

Rhesus monkeys are already more closely related to humans compared to mice and other animals but they still do not fully mimic asthma as is present in humans⁴⁶. With all these animals having different limitations and the ethical and logistical limitations of human experiments alternative models are needed. *In vitro* models are also available and have been proven useful in understanding specific cellular mechanisms but these models are even further detached from the *in vivo* scenario, lacking interplay of cells from the entire body.

With the recent technological advancements there are other options emerging to study disease progression, new techniques are being improved and optimized which might become a more suitable model, such as precision cut lung slices and tissue engineering. These techniques do currently still have some limitations. Current limitation of precision cut lung slices is the inability of using human asthmatic tissue. But new advances in this field are being made and if these advances lead to the possibility of using human tissue this might lead to a novel and promising model, allowing us to study specific pathological pathways and cell types involved in asthma. Precision cut lung slices of human tissue would also be a suitable model for preclinical drug screening with a higher translational efficacy. However this model would only be suitable to identify new pathways and for the screening of drugs and will not be a potential model for immunotherapy.

Finally, tissue engineering is rapidly evolving and even though further advancements in this field are necessary, recently there have been promising attempts to create microfluidic models of the lungs⁵³. This technique might bring a good alternative model, especially for preclinical drug screening compared to current mouse models⁵⁴. These models have many advantages compared to mouse models, they are cheaper, can be automated, can measure different parameters in real-time, and have many possible modification to suit your needs⁵⁵. This model also has the limitation that it is unable to modulate immune response and thus not a suitable model for immunotherapy. Table 1, shows a brief overview of the advantages and disadvantages of the different experimental models.

As stated before, the last few mentioned models (tissue engineering and precision cut lung slices) are promising models suitable for unraveling new pathways and potential targets but they are not able to study the immune responses like the mouse models can. Due to earlier stated limitations of other animal models; mainly ethical, logistical, and economical. Mouse models will still be the go-to immunotherapy model for allergic asthma. As previously stated, there are many different variations of this model, and the model used should be determined based on research goals. For the most clinically relevant mouse model, a chronic BALB/cJ mouse model of HDM; grass pollen; or another allergen should be used which is relevant to the human allergic asthma development. In these models an adjuvant should not be added for sensitization and a clinically relevant type of administration should be used. This is merely a suggestion, and depending on research goals other strains, different allergen administrations, and different allergens could be used.

Even though mouse models are still the go-to option, caution is advised regarding comparing and extrapolating data from these studies because of the large amount of different protocols and the lacking translational efficacy.

Table 1. Advantages and disadvantages of different experimental models

Type of model	Advantages	Disadvantages
Rodent models - Mice - Rats - Guinea pigs	- Easy to use - Relatively cheap - Good for early research	- Lacking translational efficacy - Genetically not closely related to humans - Do not mimic asthma as in shown in humans
Non-primate, non-rodent models - Cats - Horses	- Better translational efficacy - Can naturally develop form of asthma	- More expensive - Housing problems - Genetically distant to humans
Primate, non-human models - Rhesus monkeys	- decent translational efficacy - Genetically more closely related to humans	- More expensive - Ethically not suitable for early new research
Human models	- Great translational efficacy - Can naturally develop asthma	- Ethically not possible to do the required <i>in vivo</i> experiments
Precision cut lung slices	- Good translational efficacy - Good for preclinical drug screening	- Technique not yet advanced enough to use human tissue - Unable to be used for immunotherapy research
Tissue engineering	- Good translational efficacy - Relatively cheap - Can be automated - Many possible modifications possible	- Technique not yet advanced enough to study novel pathological pathways - Unable to be used for immunotherapy research

Acknowledgements

I would like to thank Laura Hesse MSc, PhD candidate at the department of Pathology and Medical Biology and dr. ir. Martijn Nawijn for the help and feedback provided.

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