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*Review Article*

# Murine Models of Allergic Asthma: Methodological Insights into Allergen Sensitization and Challenge Protocols

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**ABSTRACT**
Asthma represents a chronic inflammatory airway disease with a steadily increasing global prevalence in recent decades. Animal models have proven invaluable in elucidating the underlying disease mechanisms and identifying innovative therapeutic approaches. The murine model is extensively used to investigate key characteristics of allergic asthma, including airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling. Classic protocols involving sensitizing and challenging animals with different types of allergens and modes of administration are major factors in inducing asthmatic features in a mouse model. The present review critically analyzes the commonly used sensitization and allergen challenge protocols for inducing acute and chronic inflammation in the airways of mouse models of asthma, emphasizing their potential in advancing therapeutic development for allergic asthma studies.
**Key words:** Asthma, acute mouse model, chronic mouse model, sensitization, and challenge

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## INTRODUCTION

Asthma affects approximately 300 million people globally and continues to exhibit a rising trend every year[1]. Its intricate nature arises from a complex interplay of genetic and environmental factors[2]. Allergic asthma, the typical phenotype in clinical asthma, is triggered by allergen exposure, manifesting as a chronic inflammatory disorder affecting the airways. Key features of asthma include airway inflammation, eosinophilia, goblet cell hypersecretion, airway hyperresponsiveness (AHR), and airway remodeling[3].

The cellular and biochemical processes underlying the development of allergic airways, associated with airway inflammation and remodeling, have been investigated in clinical and animal studies[4]. Studying asthma in humans is ethically challenging, although it is the best approach to understand the pathophysiology of the disease and to investigate drug efficacy for new drug development in allergic asthma. Hence, the utilization of animal models is essential for a comprehensive understanding of the disease, notwithstanding their limitations in replicating the complexity of human asthma.

The mouse model is widely employed to investigate the involvement of various cells and mediators, as well as structural and physiological manifestations of allergic asthma progression. This review focuses on the establishment of allergic asthma, incorporating different types of allergens and administration methods during sensitization and challenge in an asthmatic mouse model.

## ALLERGIC-INDUCED TYPE 2 EOSINOPHILIC ASTHMA

In general, asthma is categorized into type 2 and non-type 2 inflammation based on distinct endotypes (**Figure 1**). Airway inflammation in type 2 immune response-driven asthma is phenotypically expressed as eosinophilic asthma, while non-type 2 immune response-driven asthma is characterized as neutrophilic asthma and paucigranulocytic asthma[5].

Eosinophilic asthma is marked by increased eosinophil production and infiltration in the airways in response to an allergen. In type 2 immune response-driven asthma, the increase in T helper 2 (Th2) lymphocytes in the peripheral blood of asthmatic patients during an exacerbation is related to the severity of airway eosinophilia, contributing to the pathophysiological changes that require aggressive treatment[6]. Upon contact with allergens presented by antigen-presenting cells (APCs) in the airway, Th2 cells secrete Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13, which recruit inflammatory cells (including eosinophils, basophils, and mast cells) and activate B cells to release immunoglobulin E (IgE) (**Figure 2**)[7].

IL-13 targets goblet cells, leading to excessive mucus production and goblet cell hyperplasia; it also

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induces eosinophil infiltration by priming the vessel wall, resulting in AHR[8]. IL-5 participates in the development, activation, and migration of eosinophils from the bone marrow to the airways, initiating airway inflammation[9]. IL-4 initiates IgE isotype class switching in B cells and upregulates the IgE receptor (Fc$\varepsilon$RI) on the mast cell surface, resulting in the release of histamine and other mediators[3]. Another hallmark of asthma is the elevated level of serum IgE synthesized by plasma cells activated by IL-4-induced class switching of B cells[10].

## MOUSE STRAIN IN THE ALLERGIC ASTHMA MOUSE MODEL

Mouse strains exhibit diverse capabilities in manifesting specific diseases and play a major role in ensuring the successful development of intended phenotypes. The most widely preferred strains include *BALB/c*, *C57BL/6*, and *A/J* mice[11].

The *BALB/c* strain has become particularly prominent in asthma studies involving allergen challenge due to its proficiency in activating a robust type 2 immune response[12]. This includes the production of Th2 cytokines, allergen-specific IgE, eosinophilic responses, and AHR. Upon allergen exposure, *BALB/c* mice readily produce these Th2 cytokines and develop AHR and airway inflammation, characterized by eosinophilic infiltration—all of which are crucial to the pathogenesis of allergic asthma[13,14]. Furthermore, their strong tendency to produce IgE antibodies in response to allergens facilitates the sensitization phase of allergic asthma. Upon allergen re-exposure, the crosslinking of IgE to mast cells subsequently triggers degranulation and the release of inflammatory mediators[15,16]. Moreover, *BALB/c* mice exhibit airway remodeling, demonstrating their capability to express the pathophysiology of the inflammatory process in asthma[17].

In contrast, the *C57BL/6* strain is regarded as a prototypic non-type 2 mouse strain, eliciting Th1 cytokines (interferon-gamma (IFN-$\gamma$) and tumor necrosis factor alpha (TNF-$\alpha$)) in response to allergen challenge[18]. Despite limitations in allergic airway development—particularly in IgE expression and AHR to methacholine—this strain is widely employed as a genetically modified animal model for assessing the impact of genetic manipulation on disease progression, including evaluation of allergen sensitization responsiveness and allergic airway inflammation[19]. Researchers also utilize other strains, such as *A/J*, in mouse models of asthma, demonstrating effectiveness in inducing AHR and increasing cytokine production[20].

Nevertheless, mouse models of asthma do not perfectly recapitulate the complexity of human asthma, largely due to the heterogeneous nature of the disease with various phenotypes. Modeling the full spectrum of human asthma in a single mouse is challenging, often necessitating a focus on specific mechanisms, such as Th2-mediated inflammation and AHR[21]. Significant differences exist between mouse and human airway anatomy and physiology, including variations in size, structure, and branching patterns that affect allergen delivery and the development of airway inflammation and remodeling[22]. While the mouse and human immune systems share similarities, genetic variations lead to significant differences, particularly in cytokine profiles, receptor expression, and gene regulation, which influence asthma development and progression[23]. Furthermore, artificial allergen sensitization protocols commonly used in mouse models—often involving repeated exposure to high doses of purified allergens—differ from natural human allergen exposure, which is typically more chronic and involves a complex mixture of allergens[24].

## ALLERGENS USED TO INDUCE ASTHMA

An allergen is any substance recognized as foreign by the immune system, provoking an allergic response. Different types of allergens can induce asthmatic conditions in animal models, with ovalbumin (OVA) being a commonly employed allergen. Whether in acute or chronic models, OVA offers advantages such as affordability, availability, a highly purified antigen, well-defined major histocompatibility complex (MHC) epitopes, and the existence of a recombinant peptide, making it a popular choice[25]. The allergic reaction induced by OVA produces a rapid, strong, and standardized response. The OVA-sensitized and challenged mouse models have successfully elucidated the effects of inflammatory cell infiltration, Th2 cytokine secretion, eosinophil recruitment, AHR, and airway remodeling[26]. Additionally, some studies have reported goblet cell hyperplasia, increased mucus production, collagen deposition, and fibrosis[27].

While the OVA model has greatly contributed to understanding the mechanisms of allergic asthma, concerns persist regarding its clinical relevance. Challenges in using OVA-induced asthma models include

**Figure 1**: **Phenotypes, endotypes, biomarkers, and characteristics of type 2 and non-type 2 asthma**. This figure outlines the classification of asthma into Type 2 and Non-Type 2 phenotypes, highlighting their distinct endotypes and associated biomarkers. Type 2 asthma, including eosinophilic subtypes and asthma with airway hyperreactivity and remodeling, is characterized by Th2 cytokines, ILC2, mast cell mediators, IgE, and epithelial-derived cytokines. Non-Type 2 asthma encompasses neutrophilic and paucigranulocytic inflammation, with neutrophil-related biomarkers. **Abbreviations**: **IL (IL-4, IL-5, IL-13, IL-17, etc.)**: Interleukin; **ILC(s) (ILC1, ILC2, ILC3)**: Innate lymphoid cell(s); **IgE**: Immunoglobulin E; **Th (Th1, Th2, Th17)**: T helper cells; **IFN-γ**: Interferon-gamma

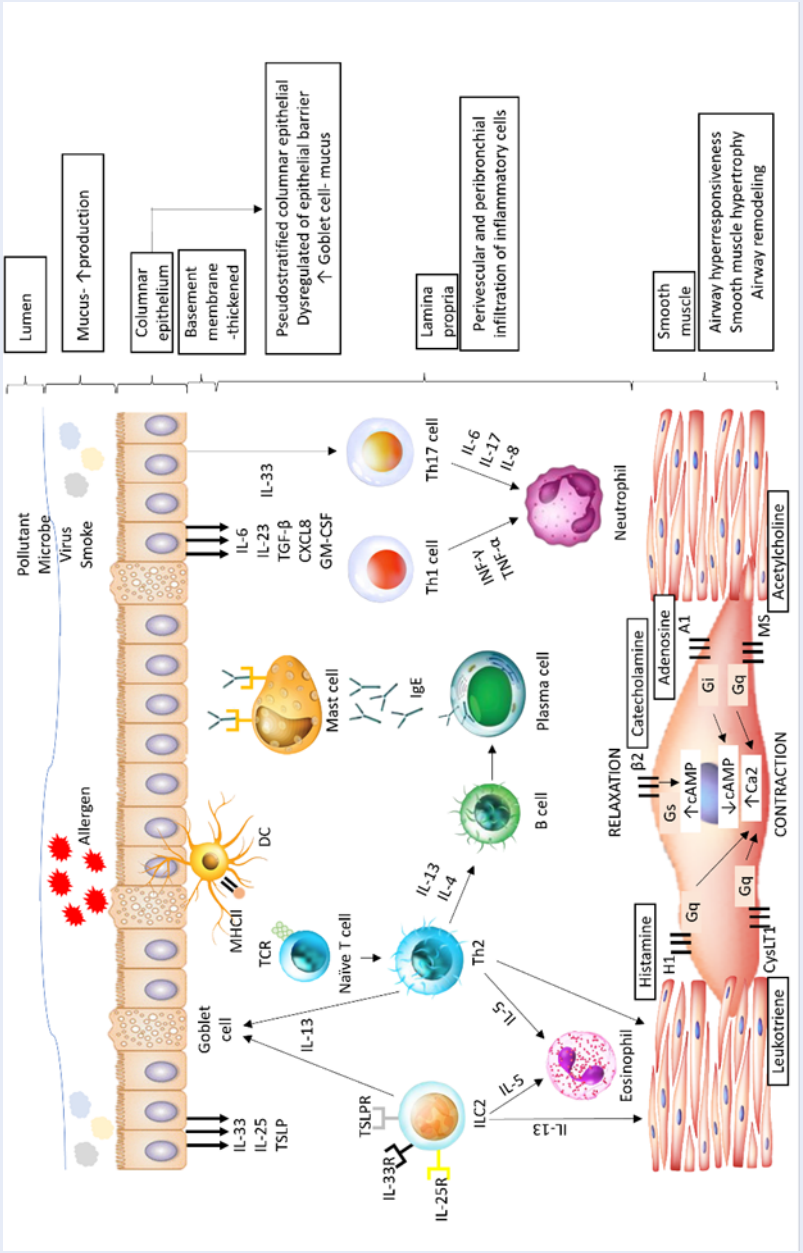


**Figure 2**: **The orchestrated pathways of asthma exacerbation involve inflammatory cell infiltration, cytokine production, AHR, and airway remodeling**. This diagram illustrates the immune mechanisms involved in asthma. Allergens and other stimuli trigger the release of cytokines from the epithelium, activating ILC2 and Th2 cells. These cells promote eosinophil recruitment and IgE production by plasma cells, leading to mast cell degranulation and the release of histamine and leukotrienes, causing airway inflammation and smooth muscle contraction. Non-Th2 pathways involving Th1 and Th17 cells and neutrophils also contribute to inflammation and airway remodeling. **Abbreviations**: **IFN-γ**: Interferon-gamma, **IgE**: Immunoglobulin E, **IL (IL-4, IL-5, IL-13, IL-17, etc.)**: Interleukin, **ILC(s) (ILC1, ILC2, ILC3)**: Innate lymphoid cell(s), **Th (Th1, Th2, Th17)**: T helper cells, **TNF-α**: Tumor necrosis factor alpha, and **TSLP**: Thymic stromal lymphopoietin

the development of OVA tolerance during long-term interventions in chronic models, the discrepancy between the human (airway) and mouse (intraperitoneal) sensitization routes—which may bypass the innate airway immune environment—and the rarity of encountering OVA in human asthma[28]. Consequently, other models using allergens more closely related to human asthma, such as house dust mite (HDM), have been developed.

Dermatophagoides farinae (American HDM) and Dermatophagoides pteronyssinus (European HDM) are common aeroallergens known to cause allergic sensitization[29]. HDM inhalation triggers pattern recognition receptors (PRRs) on airway epithelial cells, leading to chemokine and cytokine secretion that cause damage to the airway epithelia[30]. The allergenicity of HDM depends on its allergenic protein load, reflected by the IgE-binding complex pattern measured by the antibody titer.

HDM sensitization and challenge in mouse models have successfully reproduced asthmatic features[31]. Immunotherapy with purified natural *D. pteronyssinus* reduced AHR, eosinophilia, and Th2 cytokines in mice, indicating potential clinical effects[32]. Der p 2.1 peptide treatment has demonstrated the ability to suppress Th2 and Th17 cell polarization via IL-10-secreting dendritic cells[33]. Derp2-FlaB fusion protein, used as a treatment in HDM-sensitized mice, inhibited AHR, eosinophil infiltration, and Derp2-specific IgE, suggesting promise as a vaccine in asthma therapy[34].

Additionally, other allergens have also been used for sensitization and challenge in asthmatic mouse models. Acute allergic inflammation induced by papain was observed to stimulate eosinophilia[9]. Intratracheal challenge with *Schizophyllum* commune fungus in an OVA-induced model increased airway neutrophilia and the secretion of IL-17A and IL-17F[35]. Coal fly dust used to sensitize *BALB/c* mice enhanced neutrophil and other inflammatory cell infiltration, as well as increased cytokine secretion[36]. Sensitization and challenge in mice using shrimp tropomyosin resulted in eosinophilia, increased IgE secretion, lung inflammation, mucus hypersecretion, goblet cell hyperplasia, collagen deposition, and dense smooth muscle, indicating that shrimp tropomyosin can be employed as an allergen to study asthma pathogenesis[37].

## ALLERGEN SENSITIZATION IN MOUSE MODELS

Sensitization procedures are essential for inducing asthmatic conditions in animal models. Since asthma does not naturally develop in mice, sensitization is necessary to introduce the allergen and requires multiple re-exposures to evoke the allergic reaction. The initial exposure to the allergen stimulates T lymphocytes to secrete Th2 cytokines, while B lymphocytes undergo isotype switching, generating allergen-specific IgE[38]. Subsequent re-exposures lead to the cross-linking of basophils and IgE-bound mast cells, triggering degranulation and the release of inflammatory mediators.

Allergens are commonly used to induce allergic responses in animal models, together with adjuvants to enhance the immunogenicity of the allergen and further support the development of asthmatic animal models[39]. OVA, HDM, and Aspergillus are clinically relevant allergens in humans and are commonly used in allergic asthma mouse models. OVA, a protein allergen mainly found in chicken's egg white, is widely used in the majority of studies on allergic asthma[40]. Various routes of sensitization, including intraperitoneal (i.p.), subcutaneous (s.c.), intranasal (i.n.) injection, and epicutaneous (ec), can be used to induce asthmatic conditions.

In the development of animal allergic asthma models, an adjuvant is administered to enhance the sensitization mechanism of allergens during the sensitization phase. Aluminum hydroxide (alum), frequently used as an adjuvant, induces a strong type 2 immune reaction[41]. The aggregate structure of alum continuously releases antigen, promoting phagocytosis and inducing local inflammation, resulting in macrophage activation, MHC class II expression, and antigen presentation[42]. The recruitment of macrophages and dendritic cells was observed in the alum-adjuvant group, with increased eosinophilic infiltration, Th2 cytokines, and IgE levels[43].

In contrast, Complete Freund's Adjuvant (CFA) induces Th17 and Th1 cell activation, resulting in neutrophilic infiltration of the lungs[44]. A few studies have reported significant neutrophil infiltration and low eosinophil numbers, indicating that CFA is effective in inducing neutrophilic asthma[45]. The lungs were also dominated by dendritic cells, macrophages, and activated B cells, with increases in the Th1 cytokine IFN$\gamma$ and the Th17 cytokine IL-17A[43]. Interestingly, different allergens administered with the same adjuvant produced different effects, where subcutaneous injection of OVA/CFA showed neutrophilic inflammation[46], whereas HDM/CFA exhibited mixed eosinophilic-neutrophilic inflammation[47]. This difference is possibly due to the distinct nature of the antigens and how they interact with the immune system. When

combined with strong adjuvants like CFA, OVA, a relatively simple protein, may preferentially stimulate a robust Th1 immune response[48], whereas HDM, a complex mixture of proteins, can activate a broader immune response, engaging both Th2 and Th17 cells[49].

Meanwhile, lipopolysaccharide (LPS) is widely used to induce mixed eosinophilic and neutrophilic inflammation in asthmatic mouse models[50]. LPS activates toll-like receptor 4 (TLR4) on lung epithelial cells, transducing a pro-inflammatory signaling pathway[51]. The concentration of inhaled LPS during sensitization determines the type of inflammation, where low levels of LPS lead to Th2 responses, while high levels induce Th1 responses[52].

Nevertheless, the use of adjuvants can alter experimental animal behavior by causing distress and interfering with the study of adjuvant-containing drugs, such as allergen-specific immunotherapy for allergy vaccine development[53]. Hence, adjuvant-free sensitization offers a more realistic model, mirroring chronic asthma manifestation in humans[54]. Adjuvant-free sensitization via subcutaneous injection can induce AHR, airway remodeling, increased IgE secretion, and eosinophil and lymphocyte infiltration[55]. Likewise, the intranasal route can induce allergic inflammation associated with Th2 cytokine secretion, increased inflammatory cell infiltration, and mucus hypersecretion[53].

Therefore, the route of sensitization, as well as the types of adjuvants and allergens used, play pivotal roles in inducing different phenotypes of asthma inflammation. The presence of various adjuvants in allergen sensitization leads to different inflammatory responses in the asthmatic airway (**Table 1**).

## ALLERGEN CHALLENGE IN MOUSE MODELS

The capability of mouse models to induce the asthmatic condition is well-established, and these models are useful for controlling inflammation. The acute allergic airway inflammatory model is predominantly studied due to its ability to successfully establish many asthmatic features. However, this acute model falls short in developing other major features observed in human asthma, such as collagen deposition and chronic airway remodeling. Consequently, the field has shifted toward developing and studying chronic allergic airway inflammation models to address the limitations of the acute model.

### Acute allergen challenge model

Because mice do not naturally develop asthma, human intervention is necessary to induce artificial asthmatic conditions in the airways. Asthma is characterized by multiple phenotypes and cannot be entirely replicated by a single model. Hence, specific phenotypes are developed depending on the objectives of the study. **Table 2** provides a summary of different sensitization and challenge protocols in acute asthmatic mouse models.

The development of an asthmatic model in mice depends on several factors, including the protocol of sensitization and challenges, the adjuvants, and the type of allergens. In the acute mouse asthma model, diverse yet coherent protocols were employed. Allergen sensitization via systemic delivery into the circulatory system commonly necessitates multiple re-exposures to establish a favorable allergic model[38]. Meanwhile, allergen challenge is usually administered via the airways through inhalation (aerosol), intratracheal (i.t.), or intranasal (i.n.) routes. The common acute model protocol involves allergen sensitization lasting for two to three weeks, followed by allergen challenge for several consecutive days, with the endpoint assessed 24 hours after the last challenge.

The acute mouse model develops the common characteristics of clinical asthma. Studies have shown that lung pathology induced by allergens can exhibit changes in the lungs that cause airway inflammation, airway remodeling, and AHR[66?]. Histological analysis allows examination of inflammatory cell recruitment, mucus production, collagen deposition, and fibrosis in the perivascular and peribronchiolar space[67]. The acute model is also utilized to study the mechanisms of remodeling and oxidative stress associated with the signaling pathway in pulmonary asthma[68,69]. Additionally, this model has also shown the amelioration of allergic inflammation when treated with various potential suppressors, such as IL-38[70], anti-IL-25[71], and leukotriene B4 receptor blocker[72].

While the acute model has successfully investigated some features of the pathophysiology of asthma, it has limitations compared to clinical asthma, which requires persistent airway inflammation to mimic asthmatic individuals. The short period of allergen challenge is one reason for minimal changes in airway remodeling, AHR, and eosinophilia, with these changes subsiding a few weeks after the last challenge. Asthma is associated with chronic disease, so some concerns arise regarding the reliability of acute mouse models in investigating disease progression and potential treatments.

**Table 1: The route of allergen and adjuvant sensitization and its effect in asthma development**

| Allergen | Adjuvar | Strain | Route | Efficacy | Reference |
|---|---|---|---|---|---|
| OVA | Alum | *BALB/c* | i.p. | ↑ eosinophils and B cells population<br>↓ GATA3 and ILC2s in LN<br>↓ IFN-$\gamma$ and Th1 cells in lung<br>↑ IL-5 and IL-4 and Th2 cells in lung and LN | [52] |
| | LPS | *BALB/c* | i.p. | ↓ eosinophils percentage<br>↑ neutrophils population in BALF<br>↑ T-bet and ILC1s in lungs<br>↑ RORγt and ILC3s in LN<br>↑ Th17 cells in lungs and LN | |
| OVA | CFA | *C57BL/6* | i.p. | ↑ neutrophils and macrophages in BALF<br>↑ inflammatory cells infiltration and goblet cells based on H&E and PAS staining<br>↑ S100A9, caspase-1, IL-1$\beta$, IL-17, IFN-$\gamma$, TNF-$\alpha$ and myeloperoxidase proteins in western blot analysis | [45] |
| OVA | CFA | *C57BL/6* | i.p. | ↑ plasmacytoid dendritic cells, exudate macrophages, and B cells<br>↑ neutrophils in BALF and lung<br>↑ Th1 cytokine IFN-$\gamma$ | [43] |
| | Alum | *C57BL/6* | i.p. | ↑ interstitial macrophages and myeloid dendritic cells<br>↑ eosinophils in BALF and lungs<br>↑ IL-5 and IL-13<br>↑ basophils and mast cells in lung tissue | |
| OVA | Alum | *BALB/c* | i.p. | ↑ eosinophils number<br>↑ IL-4, IL-5, IL-13 and IL-33 in BALF<br>Moderate inflammation (only bronchi and vessels of the lungs infiltrated with inflammatory cells) | [58] |
| | LPS | *BALB/c* | i.p. | ↑ neutrophils number<br>↑ Th1 (IFN-$\gamma$) and Th17 (IL-17A) in BALF<br>Severe inflammation (nearly whole lung infiltrated with inflammatory cells) | |
| HDM | Alum | *BALB/c* | s.c. | ↑ IgE level<br>↑ Th2 cytokines | [56] |
| HDM | CFA | *C57BL/6* | s.c. | ↑ macrophage MIF in BALF<br>↑ mixed eosinophilic/neutrophilic response<br>AHR | [47] |
| OVA | CFA | *BALB/c* | s.c. | ↑ neutrophils count<br>↑ inflammatory cell infiltration<br>AHR | [46] |
| OVA | LPS | *BALB/c* | i.n. | ↑ Th2 (IL-4, IL-5, IL-13) and Th17 (IL-17)<br>↓ Th1 (IFN-$\gamma$) and Treg (TGF-$\beta$, IL-10)<br>↑ GATA3, T-bet, and ROR-$\gamma$t expression<br>↓ T-bet, Foxp3 and IL-10 expression<br>AHR | [57] |

**Abbreviations**: **i.p.**: intraperitoneal; **s.c.**: subcutaneous; **i.n.**: intranasal; **ILCs**: innate lymphoid cells; **LN**: lymph node; **IFN-$\gamma$**: interferon-gamma; **Th**: T helper cells; **IL**: interleukin; **BALF**: Bronchoalveolar lavage fluid; **T-bet**: T-box transcription factor TBX21; **RORγt**: retinoic acid receptor-related orphan receptor gamma t; **H&E**: hematoxylin and eosin; **PAS**: periodic acid-schiff; **S100A9**: S100 calcium-binding protein A9; **TNF-$\alpha$**: tumor necrosis factor alpha; **IgE**: immunoglobulin E; **MIF**: migration inhibitory factor; **AHR**: airway hyperresponsiveness; **Treg**: regulatory T cells; **TGF-$\beta$**: transforming growth factor-beta; **Foxp3**: forkhead box protein 3.

**Table 2: Acute allergic airway inflammation in acute asthmatic mouse models**

| Strain/ge | Allerger | Sensitization/ro | Challenge/rou | Responses to challenge | References |
|---|---|---|---|---|---|
| *BALB/c* Female | OVA | Day 0 and 7 OVA + alum i.p. | Day 14-18 OVA i.n. | AHR and airway inflammation | [59] |
| *BALB/c* Female | HDM | Day 0 and 7 HDM + alum i.p. | Day 14-25 HDM i.n. | AHR, inflammatory cells infiltration, eosinophilia, Th2 cytokines and IL-33 secretion | [60] |
| *BALB/c* Male | OVA | Day 0 and 14 OVA + alum i.p. | Day 21-23 OVA Aerosol | Neutrophils and eosinophil infiltration airway wall thickening | [52] |
| *C57BL/6* Female | OVA | Day 0 and 5 OVA + alum i.p. | Day 12 and 13 OVA Aerosol | Neutrophilia and airway inflammation | [35] |
| *BALB/c* Female | OVA | Day 0 and 14 OVA + alum i.p. | Day 28-30 OVA Aerosol | Inflammatory cells infiltration, Th2 cytokines secretion, eosinophilia | [61] |
| *BABL/c* Male | OVA | Day 7 and 14 OVA + alum i.p. | Day 21-23 OVA Aerosol | Leukocytes infiltration, eosinophilia and TNF-$\alpha$, IL-1$\beta$, IL-6, TGF-$\beta$, and IFN-$\gamma$ secretion | [62] |
| *Balb/c* Male | OVA | Day 0, 2, 4, 7, 9 and 10 OVA i.p. | Day 15, 18 and 21 OVA i.t. | Inflammatory cells infiltration, muscle and epithelial thickening, epithelial desquamation, goblet cell metaplasia, and collagen deposition | [17] |
| *BALB/c* Female | OVA | Day 1 and 14: OVA + alum i.p. | Day 25-28 OVA i.n. | Inflammatory cells inflammation and IL-5 and IL-13 secretion | [63] |
| *C57BL/6* Female | OVA | Day 1 and 15 OVA + alum i.p. | Day 21-23 OVA Aerosol | Aberrant miRNAs profile in the $CD4^+$ T lymphocytes | [64] |
| *BALB/c* Female | OVA | Day 1, 8 and 15 OVA + alum i.p. | Day 16-22 OVA Aerosol | Airway inflammation and remodeling, inflammatory cells infiltration and Th2 cytokines secretion | [65] |

**Abbreviations**: **i.p.**: intraperitoneal; **i.n.**: intranasal; **i.t.**: intratracheal; **OVA**: ovalbumin; **HDM**: house dust mite; **alum**: aluminium hydroxide; **AHR**: airway hyperresponsiveness; **Th**: T helper cells; **IL**: interleukin; **TNF-$\alpha$**: tumor necrosis factor alpha; **TGF-$\beta$**: transforming growth factor-beta; **IFN-$\gamma$**: interferon-gamma; **miRNAs**: micro ribonucleic acids

## Chronic allergen challenge model

A chronic mouse model with prolonged allergen challenges overcomes several issues encountered in the acute mouse model. Significant differences in AHR, airway remodeling, and inflammatory profiles between acute and chronic asthmatic models have been observed in clinical asthma. The chronicity of allergen exposure is a critical concern in the acute model, as the sensitization and challenge procedures may not induce persistent changes in airway inflammation, unlike in humans. Various chronic sensitization and challenge protocols have been employed, with some summarized in **Table 3**.

Chronic allergen challenge contributes to persistent airway remodeling, depicted by collagen deposition, airway inflammation, goblet cell hyperplasia, and eosinophilia in the mouse model[73,74]. The chronic model typically spans 4 to 12 weeks, starting with allergen sensitization followed by repeated low-level allergen exposure. Different types of allergens have been used to simulate the chronic model, and adjuvant-free protocols have been employed to imitate the natural sensitization that occurs in hu-

mans[75].

The presence of T cells is essential for an immediate response to recurrent allergen exposure[76]. Chronic allergen exposure has demonstrated a $CD4^+$ and $CD8^+$ T cell-dependent effect on airway inflammatory cell infiltration and AHR[75,77]. Moreover, eosinophils play an important role in the remodeling process by altering the structure of airway nerves, inducing AHR and fibrosis, and thereby increasing allergen sensitivity in eosinophilic asthma associated with chronic allergen exposure[78]. Extensive research using chronic murine asthma models has explored the roles of some proteins, such as the WNT5A ligand[79], microRNA-221[80], and IL-33[73], to understand their effects on asthma pathogenesis.

The chronic mouse model has successfully replicated key features of human asthma and is currently employed to study potential therapeutic treatments applicable at the clinical stage. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells have shown therapeutic potential in the chronic asthma model, particularly in a hypoxic environment[81]. This study demonstrated significant attenuation of airway inflammation, represented by the depletion of inflammatory cells, eosinophils, and Th2 cytokines, and amelioration of airway remodeling, accompanied by decreases in alpha-smooth muscle actin ($\alpha$-SMA), collagen type 1, and transforming growth factor-beta (TGF-$\beta$) 1 signaling pathway expression.

Additionally, this model is also used to gain a better understanding of biochemical changes within complex tissue samples of potential anti-asthmatic compounds[82]. Novel imaging techniques that combine the analytical approaches of focal plane array (FPA) and synchrotron Fourier-transform infrared (S-FTIR) enable the investigation of broader molecular changes surrounding the airways and identification of types of collagen deposition present in the chronic asthma model, further supporting the analysis of conventional methods.

However, several hindrances related to the chronic mouse model were identified when compared to human asthma. In humans, asthma often develops spontaneously in early life alongside immature lung development, compared to the fully developed lungs of mice at birth, necessitating artificial allergen and adjuvant sensitization[83]. The route, amount, and frequency of allergen exposure in controlled conditions of allergic airway mouse models differ from the natural and acquired immune responses of asthma exacerbation in humans and do not reflect patient heterogeneity[84,85].

Moreover, the extended period of inhaled antigen exposure in mice induces tolerance, described by changes in inflammatory cell profiles, airway inflammation, and AHR, limiting the opportunities to investigate the chronic model and the underlying pathways[86,87]. Nevertheless, allergen tolerance provides some advantages for studying the effect of certain parameters associated with asthma for therapeutic development. Inhaled allergens may induce an inappropriate Th2-cell inflammatory response, and this adverse reaction can be obscured via the local inhalation tolerance process to restore airway homeostasis[88] and regulation of free IgE[89], thereby diminishing asthma symptoms.

While invaluable for research, these chronic mouse models pose significant ethical challenges. Prolonged suffering, due to repeated allergen exposure leading to chronic inflammation, AHR, and airway remodeling, can cause discomfort, breathing difficulties, and potentially pain over extended periods[90]. Assessing pain and distress can be challenging, as subtle behavioral changes may indicate underlying suffering but are difficult to interpret definitively[91]. Therefore, researchers must carefully optimize research protocols by minimizing the duration and intensity of allergen exposure, balancing the need to reduce distress with the requirement to obtain meaningful data. Animals should also be monitored regularly for signs of distress, including routine assessment of respiratory function and behavior.

## FUTURE PERSPECTIVES AND CONCLUSION

Allergen sensitization and challenge in mouse models represent classical protocols for manifesting asthma pathophysiology. Researchers are striving to model specific disease phenotypes that accurately replicate the complex nature of human asthma. While acute allergen challenges effectively represent several hallmarks of asthma, they fall short of capturing certain features of chronic asthma. Therefore, the development of chronic allergen challenge models aims to deepen understanding of disease mechanisms and discover novel therapeutic potentials.

Allergic mouse models require active sensitization, typically introduced with adjuvants administered intraperitoneally or subcutaneously alongside the allergen. These methods are less intrusive and do not require sedation, but they may induce tolerance. As a result, models without adjuvants have been developed to induce sensitization in the airways via intranasal instillation, simulating the natural exposure of humans to airborne allergens. This model has

**Table 3: Chronic allergic airway inflammation in chronic asthmatic mouse models**

| Strain/gen | Allergen | Sensitization/ro | Challenge/route | Response to challenge | References |
|---|---|---|---|---|---|
| *BALB/c* Female | OVA | Day 0, 7, and 14<br>OVA + alum<br>i.p. | Day 21-55<br>OVA<br>Aerosol/i.n. | Airway remodeling, inflammatory cells infiltration, eosinophilia, increased mucus production and IL-4 and IL-13 secretion | 82 |
| *BALB/c* Female | OVA | Day 0, 7, 14, and 21<br>OVA + alum<br>s.c. | Days 33 and 35:<br>OVA<br>i.n. | AHR, airway inflammation, and remodeling, Th2 cytokines, TSLP, IL-33 and IL-25 secretion, goblet cell hyperplasia, increased TNF-$\alpha$, and collagen deposition | 92 |
| *BALB/c* Female | OVA | Day 0, 14, 28 and 42<br>OVA + alum<br>i.p. | Day 21-42<br>OVA<br>Aerosol | Airway remodeling, inflammatory cells infiltration, elevated IgE, IL-6 and IL-13 | 93 |
| *Balb/c* Female | HDM | Days 0, 7, and 14<br>HDM<br>i.p. | Day 21–28<br>HDM<br>i.n. | Inflammatory cells infiltration, Th2 cytokines secretion and specific IgE production, airway wall thickening, mucosal metaplasia, collagen deposition, goblet cell hyperplasia and mucus hypersecretion. | 30 |
| *BALB/c* Female | OVA | Day 1 and 14<br>OVA + alum<br>i.p. | Day 14, 17, 21, 24, 27, 60, 69, 71, 73, 74, and 75<br>OVA<br>i.n. | Inflammatory cells infiltration, Th2 cytokine, IL-17, TNF-$\alpha$ and high mobility group box protein 1 secretion. | 94 |
| *BALB/c* Female | OVA | Day 1, 2 and 3<br>OVA + alum<br>i.p. | Day 14, 17, 21, 24, 27, 60, 69, 71, 73, 74, and 75<br>OVA<br>i.n. | Airway remodeling, inflammatory cells infiltration and Th2, Th1, IL-17 and IL-22 cytokines secretion and collagen deposition | 95 |
| *BALB/c* Female | OVA | Day 1 and 14<br>OVA + alum<br>i.p. | Day 28, 30, 32, 34, 36, 38, 40, 42 and 44<br>OVA<br>Aerosol | Airway inflammation, fibrotic airway remodeling and inflammatory cells infiltration | 63 |
| *C57BL/6* Female | HDM | Day 1<br>HDM<br>i.n. | Day 2-36:<br>HDM<br>i.n. | Th2-mediated eosinophilic inflammation and IL-12 and IL-6 production | 96 |
| *Balb/c* Male | OVA | Day 0 and 14<br>OVA+ alum<br>i.p. | Three times per week for 9 weeks<br>OVA<br>Aerosol | Day 87:<br>AHR, inflammatory cells infiltration, eosinophilia, and mucus hypersecretion | 97 |
| *C57BL/6* Female | HDM | Day 0 and 7:<br>HDM<br>i.n. | five times per week for three weeks, rested (4–8 week) and rechallenged<br>HDM<br>i.n. | 24 hours after the final challenge:<br>AHR, increased $CD4^+$ T cells and dendritic cells | 98 |

**Abbreviations**: **i.p.**: intraperitoneal; **i.n.**: intranasal; **s.c.**: subcutaneous; **OVA**: ovalbumin; **alum**: aluminum hydroxide; **IL**: interleukin; **Th**: T helper cells; **TLSP**: thymic stromal lymphopoietin; **TNF-$\alpha$**: tumor necrosis factor alpha; **IgE**: immunoglobulin E; **HDM**: house dust mite; **AHR**: airway hyperresponsiveness.

proven effective in producing a phenotype of asthma comparable to that of the traditional adjuvant model. In allergen challenge, aerosol and intranasal routes are likely closer to mimicking human exposure than the intratracheal approach. The allergen OVA may inadvertently induce tolerance with repeated and prolonged exposure, in contrast to HDM, which exhibits persistent airway inflammation, making it more suitable for modeling chronic asthma. Therefore, adjuvant-free models and aeroallergen exposure may be more relevant in mimicking human asthma for the development of new treatments and preventive approaches. Despite the shortcomings of both acute and chronic allergic asthma models, ongoing research aims to improve protocols to enhance our understanding of asthma at the cellular and molecular levels.

## ABBREVIATIONS

**$\alpha$-SMA** (Alpha-smooth muscle actin), **AHR** (Airway hyperresponsiveness), **Alum** (Aluminum hydroxide), **APC(s)** (Antigen-presenting cell(s)), **BALF** (Bronchoalveolar lavage fluid), **CFA** (Complete Freund's Adjuvant), **ec** (Epicutaneous), **Foxp3** (Forkhead box protein 3), **FPA** (Focal plane array, an imaging technique), **H&E** (Hematoxylin and eosin staining), **HDM** (House dust mite), **IFN-$\gamma$** (Interferon-gamma), **IgE** (Immunoglobulin E), **IL** (IL-4, IL-5, IL-13, IL-17, etc.) (Interleukin), **ILC(s)** (ILC1, ILC2, ILC3) (Innate lymphoid cell(s)), **i.n.** (Intranasal), **i.p.** (Intraperitoneal), **i.t.** (Intratracheal), **LN** (Lymph node), **LPS** (Lipopolysaccharide), **MHC** (Major histocompatibility complex), **miRNAs** (Micro ribonucleic acids), **OVA** (Ovalbumin), **PAS** (Periodic acid–Schiff staining), **RORγt** (Retinoic acid receptor-related orphan receptor gamma t), **s.c.** (Subcutaneous), **S-FTIR** (Synchrotron Fourier-transform infrared spectroscopy), **S100A9** (S100 calcium-binding protein A9), **T-bet** (T-box transcription factor TBX21), **TGF-$\beta$** (Transforming growth factor-beta), **Th** (Th1, Th2, Th17) (T helper cells), **TLR4** (Toll-like receptor 4), **TNF-$\alpha$** (Tumor necrosis factor alpha), and **TSLP** (Thymic stromal lymphopoietin)

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## AUTHOR'S CONTRIBUTIONS

Bushra Solehah Mohd Rosdan served as the primary author and was responsible for the initial drafting and subsequent editing of the manuscript. Nurul Asma Abdullah provided supervision, conducted critical reviews, and contributed to manuscript revisions. All authors have read and approved the final version of the manuscript.

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## AVAILABILITY OF DATA AND MATERIALS

Not applicable.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

1. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1789–858. PMID: 30496104. Available from: https://doi.org/10.1016/S0140-6736(18)32279-7.
2. Morales E, Duffy D. Genetics and Gene-Environment Interactions in Childhood and Adult Onset Asthma. Frontiers in Pediatrics. 2019;7:499. PMID: 31921716. Available from: https://doi.org/10.3389/fped.2019.00499.
3. Athari SS. Targeting cell signaling in allergic asthma. Signal Transduction and Targeted Therapy. 2019;4(1):45. PMID: 31637021. Available from: https://doi.org/10.1038/s41392-019-0079-0.
4. Liu Y, Wei L, He C, Chen R, Meng L. Lipoxin A4 inhibits ovalbumin-induced airway inflammation and airway remodeling in a mouse model of asthma. Chemico-Biological Interactions. 2021;349:109660. PMID: 34537180. Available from: https://doi.org/10.1016/j.cbi.2021.109660.
5. Svenningsen S, Nair P. Asthma Endotypes and an Overview of Targeted Therapy for Asthma. Frontiers in Medicine. 2017;4:158. PMID: 29018800. Available from: https://doi.org/10.3389/fmed.2017.00158.
6. Palikhe NS, Wu Y, Konrad E, Gandhi VD, Rowe BH, Vliagoftis H. Th2 cell markers in peripheral blood increase during an acute asthma exacerbation. Allergy. 2021;76(1):281–90. PMID: 32750154. Available from: https://doi.org/10.1111/all.14543.
7. Sze E, Bhalla A, Nair P. Mechanisms and therapeutic strategies for non-T2 asthma. Allergy. 2020;75(2):311–25. PMID: 31309578. Available from: https://doi.org/10.1111/all.13985.
8. Chigbu DI, Jain P, Khan ZK. Immune Mechanisms, Pathology, and Management of Allergic Ocular Diseases. In: Jain, P., Ndhlovu, L. (eds) Advanced Concepts in Human Immunology: Prospects for Disease Control. Springer, Cham. https://doi.org/10.1007/978-3-030-33946-3_4. Cham: Springer International Publishing; 2020. Available from: https://doi.org/10.1007/978-3-030-33946-3_4.

9. Boberg E, Johansson K, Malmhäll C, Calvén J, Weidner J, RM. Interplay Between the IL-33/ST2 Axis and Bone Marrow ILC2s in Protease Allergen-Induced IL-5-Dependent Eosinophilia. Frontiers in Immunology. 2020;11:1058. PMID: 32582171. Available from: https://doi.org/10.3389/fimmu.2020.01058.
10. Humbert M, Bousquet J, Bachert C, Palomares O, Pfister P, Kottakis I. IgE-Mediated Multimorbidities in Allergic Asthma and the Potential for Omalizumab Therapy. The Journal of Allergy and Clinical Immunology In Practice. 2019;7(5):1418–29. PMID: 30928481. Available from: https://doi.org/10.1016/j.jaip.2019.02.030.
11. Azman S, Sekar M, Bonam SR, Gan SH, Wahidin S, Lum PT. Traditional Medicinal Plants Conferring Protection Against Ovalbumin-Induced Asthma in Experimental Animals: A Review. Journal of Asthma and Allergy. 2021;14:641–62. PMID: 34163178. Available from: https://doi.org/10.2147/JAA.S296391.
12. Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. Cell and Tissue Research. 2017;367(3):551–69. PMID: 28190087. Available from: https://doi.org/10.1007/s00441-016-2566-8.
13. Huang WC, Wu SJ, Fang LW, Lin TY, Liou CJ. Phillyrin attenuates airway inflammation and Th2 cell activities in a mouse asthma model. Food and Agricultural Immunology. 2023;34(1). Available from: https://doi.org/10.1080/09540105.2023.2231182.
14. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. Immunity. 2019;50(4):975–91. PMID: 30995510. Available from: https://doi.org/10.1016/j.immuni.2019.03.018.
15. Gouel-Chéron A, Dejoux A, Lamanna E, Bruhns P. Animal Models of IgE Anaphylaxis. Biology (Basel). 2023;12(7):931. PMID: 37508362. Available from: https://doi.org/10.3390/biology12070931.
16. Miranda-Waldetario MC, de Lafaille MAC. Making good of a tricky start: how IgE and mast cells manage a protective sway in food allergy. Immunity. 2023;56(9):1988–90. PMID: 37703829. Available from: https://doi.org/10.1016/j.immuni.2023.08.010.
17. Maria L, Leite B, Guilherme L, Capriglione A. Histopathologic evaluation, anesthetic protocol, and physiological parameters for an experimental Balb/c mouse model of asthma. Lungs and Breathing. 2019;3(1):1–6. Available from: https://doi.org/10.15761/LBJ.1000139.
18. Cardoso FO, do Valle TZ, Almeida-Souza F, Abreu-Silva AL, Calabrese KD. Modulation of Cytokines and Extracellular Matrix Proteins Expression by Leishmania amazonensis in Susceptible and Resistant Mice. Frontiers in Microbiology. 2020;11:1986. PMID: 32983013. Available from: https://doi.org/10.3389/fmicb.2020.01986.
19. Park MK, Park HK, Yu HS. Toll-like receptor 2 mediates Acanthamoeba-induced allergic airway inflammatory response in mice. PLoS Neglected Tropical Diseases. 2023;17(1):e001108. PMID: 36706056. Available from: https://doi.org/10.1371/journal.pntd.0011085.
20. Casaro MB, Thomas AM, Mendes E, Fukumori C, Ribeiro WR, Oliveira FA. A probiotic has differential effects on allergic airway inflammation in A/J and C57BL/6 mice and is correlated with the gut microbiome. Microbiome. 2021;9(1):134. PMID: 34112246. Available from: https://doi.org/10.1186/s40168-021-01081-2.
21. Rosenberg HF, Druey KM. Modeling asthma: Pitfalls, promises, and the road ahead. Journal of Leukocyte Biology. 2018;104(1):41–8. PMID: 29451705. Available from: https://doi.org/10.1002/JLB.3MR1117-436R.
22. Rydell-Törmänen K, Johnson JR. The Applicability of Mouse Models to the Study of Human Disease. In: Bertoncello, I. (eds) Mouse Cell Culture. Methods in Molecular Biology, vol 1940. Humana Press, New York, NY; 2019. Available from: https://doi.org/10.1007/978-1-4939-9086-3_1.
23. Forbester JL, Humphreys IR. Genetic influences on viral-induced cytokine responses in the lung. Mucosal Immunology. 2021;14(1):14–25. PMID: 33184476. Available from: https://doi.org/10.1038/s41385-020-00355-6.
24. Radhouani M, Starkl P. Adjuvant-independent airway sensitization and infection mouse models leading to allergic asthma. Frontiers in Allergy. 2024;5:1423938. PMID: 39157265. Available from: https://doi.org/10.3389/falgy.2024.1423938.
25. Mostashari P, MarszaK, Aliyeva A, Khaneghah AM. The Impact of Processing and Extraction Methods on the Allergenicity of Targeted Protein Quantification as Well as Bioactive Peptides Derived from Egg. Molecules (Basel, Switzerland). 2023;28(6):2658. PMID: 36985630. Available from: https://doi.org/10.3390/molecules28062658.
26. Ponci V, Silva RC, Santana FP, Grecco SS, Fortunato CR, Oliveira MA. Biseugenol Exhibited Anti-Inflammatory and Anti-Asthmatic Effects in an Asthma Mouse Model of Mixed-Granulocytic Asthma. Molecules (Basel, Switzerland). 2020;25(22):5384. PMID: 33217892. Available from: https://doi.org/10.3390/molecules25225384.
27. Piao CH, Bui TT, Fan YJ, Nguyen TV, Shin DU, Song CH, et al. In vivo and in vitro anti-allergic and anti-inflammatory effects of Dryopteris crassirhizoma through the modulation of the NF-κB signaling pathway in an ovalbumin-induced allergic asthma mouse model. Molecular Medicine Reports. 2020;22(5):3597–606. PMID: 33000211. Available from: https://doi.org/10.3892/mmr.2020.11460.
28. Sethi GS, Naura AS. Progressive increase in allergen concentration abrogates immune tolerance in ovalbumin-induced murine model of chronic asthma. International Immunopharmacology. 2018;60:121–31. PMID: 29729496. Available from: https://doi.org/10.1016/j.intimp.2018.04.047.
29. Zhang J, Chen J, Robinson C. Cellular and Molecular Events in the Airway Epithelium Defining the Interaction Between House Dust Mite Group 1 Allergens and Innate Defences. International Journal of Molecular Sciences. 2018;19(11):3549. PMID: 30423826. Available from: https://doi.org/10.3390/ijms19113549.
30. Liu M, Lu J, Zhang Q, Zhang Y, Guo Z. Clara cell 16 KDa protein mitigates house dust mite-induced airway inflammation and damage via regulating airway epithelial cell apoptosis in a manner dependent on HMGB1-mediated signaling inhibition. Molecular Medicine (Cambridge, Mass). 2021;27(1):11. PMID: 33541260. Available from: https://doi.org/10.1186/s10020-021-00277-4.
31. Park SJ, Lee K, Kang MA, Kim TH, Jang HJ, Ryu HW, et al. Tilianin attenuates HDM-induced allergic asthma by suppressing Th2-immune responses via downregulation of IRF4 in dendritic cells. Phytomedicine : International Journal of Phytotherapy and Phytopharmacology. 2021;80:153392. PMID: 33113503. Available from: https://doi.org/10.1016/j.phymed.2020.153392.
32. Hesse L, van Ieperen N, Habraken C, Petersen AH, Korn S, Smilda T. Subcutaneous immunotherapy with purified Der p1 and 2 suppresses type 2 immunity in a murine asthma model. Allergy. 2018;73(4):862–74. PMID: 29318623. Available from: https://doi.org/10.1111/all.13382.
33. Klein M, Colas L, Cheminant MA, Brosseau C, Sauzeau V, Magnan A, et al. Der p 2.1 Peptide Abrogates House Dust Mites-Induced Asthma Features in Mice and Humanized Mice by Inhibiting DC-Mediated T Cell Polarization. Frontiers in Immunology. 2020;11:565431. PMID: 33312170. Available from: https://doi.org/10.3389/fimmu.2020.565431.
34. Tan W, Zheng JH, Duong TM, Koh YI, Lee SE, Rhee JH. A Fusion Protein of Derp2 Allergen and Flagellin Suppresses Experimental Allergic Asthma. Allergy, Asthma & Immunology Research. 2019;11(2):254–66. PMID: 30661317. Available from: https://doi.org/10.4168/aair.2019.11.2.254.
35. Hanashiro J, Muraosa Y, Toyotome T, Hirose K, Watanabe A, Kamei K. Schizophyllum commune induces IL-17-mediated neutrophilic airway inflammation in OVA-induced asthma model mice. Scientific Reports. 2019;9(1):19321. PMID: 31852931. Available from: https://doi.org/10.1038/s41598-019-55836-x.

36. Saba E, Lee YS, Yang WK, Lee YY, Kim M, Woo SM. Effects of a herbal formulation, KGC3P, and its individual component, nepetin, on coal fly dust-induced airway inflammation. Scientific Reports. 2020;10(1):14036. PMID: 32820197. Available from: https://doi.org/10.1038/s41598-020-68965-5.
37. Fang L, Zhou F, Wu F, Yan Y, He Z, Yuan X, et al. A mouse allergic asthma model induced by shrimp tropomyosin. International Immunopharmacology. 2021;91:107289. PMID: 33370683. Available from: https://doi.org/10.1016/j.intimp.2020.107289.
38. Sadeghi M, Koushki K, Mashayekhi K, Ayati SH, Shahbaz SK, Moghadam M, et al. DC-targeted gold nanoparticles as an efficient and biocompatible carrier for modulating allergic responses in sublingual immunotherapy. International Immunopharmacology. 2020;86:106690. PMID: 32585607. Available from: https://doi.org/10.1016/j.intimp.2020.106690.
39. Raspe J, Schmitz MS, Barbet K, Caso GC, Cover TL, Müller A. Therapeutic properties of Helicobacter pylori-derived vacuolating cytotoxin A in an animal model of chronic allergic airway disease. Respiratory Research. 2023;24(1):178. PMID: 37415170. Available from: https://doi.org/10.1186/s12931-023-02484-5.
40. Sharif M, Anjum I, Shabbir A, Syed SK, Mobeen I, Shahid MH, et al. Amelioration of Ovalbumin-Induced Allergic Asthma by Juglans regia via Downregulation of Inflammatory Cytokines and Upregulation of Aquaporin-1 and Aquaporin-5 in Mice. Journal of Tropical Medicine. 2022;2022:6530095. PMID: 35401757. Available from: https://doi.org/10.1155/2022/6530095.
41. Kim CY, Kim JW, Kim JH, Jeong JS, Lim JO, Ko JW. Inner Shell of the Chestnut (Castanea crenatta) Suppresses Inflammatory Responses in Ovalbumin-Induced Allergic Asthma Mouse Model. Nutrients. 2022;14(10):2067. PMID: 35631208. Available from: https://doi.org/10.3390/nu14102067.
42. Ko EJ, Lee YT, Kim KH, Lee Y, Jung YJ, Kim MC. Roles of Aluminum Hydroxide and Monophosphoryl Lipid A Adjuvants in Overcoming CD4+ T Cell Deficiency To Induce Isotype-Switched IgG Antibody Responses and Protection by T-Dependent Influenza Vaccine. The Journal of Immunology : Official Journal of the American Association of Immunologists. 2017;198(1):279–91. PMID: 27881702. Available from: https://doi.org/10.4049/jimmunol.1600173.
43. Özkan M, Eskiocak YC, Wingender G. Macrophage and dendritic cell subset composition can distinguish endotypes in adjuvant-induced asthma mouse models. PLoS One. 2021;16(6):e0250533. PMID: 34061861. Available from: https://doi.org/10.1371/journal.pone.0250533.
44. Menson KE, Mank MM, Reed LF, Walton CJ, Vliet KEVD, Ather JL. Therapeutic efficacy of IL-17A neutralization with corticosteroid treatment in a model of antigen-driven mixed-granulocytic asthma. American Journal of Physiology Lung Cellular and Molecular Physiology. 2020;319(4):693–709. PMID: 32783616. Available from: https://doi.org/10.1152/ajplung.00204.2020.
45. Lee JU, Park JS, Jun JA, Kim MK, Chang HS, Baek DG, et al. Inhibitory Effect of Paquinimod on a Murine Model of Neutrophilic Asthma Induced by Ovalbumin with Complete Freund's Adjuvant. Canadian Respiratory Journal. 2021;2021:8896108. PMID: 33791048. Available from: https://doi.org/10.1155/2021/8896108.
46. Zhao MZ, Li Y, Han HY, Mo LH, Yang G, Liu ZQ. Specific Ag-guiding nano-vaccines attenuate neutrophil-dominant allergic asthma. Molecular Immunology. 2021;129:103–11. PMID: 33229073. Available from: https://doi.org/10.1016/j.molimm.2020.11.005.
47. sita rama raju Allam V, Adcock I, Chung KF, Morand E, Harris J, Sukkar M. MIF antagonism restores corticosteroid sensitivity in a murine model of severe asthma. European Respiratory Journal . 2018;52(suppl 62):PA979. Available from: https://doi.org/10.1183/13993003.congress-2018.PA979.
48. Xia M, Xu F, Ni H, Wang Q, Zhang R, Lou Y. Neutrophil activation and NETosis are the predominant drivers of airway inflammation in an OVA/CFA/LPS induced murine model. Respiratory Research. 2022;23(1):289. PMID: 36271366. Available from: https://doi.org/10.1186/s12931-022-02209-0.
49. Tynecka M, Janucik A, Tarasik A, Zbikowski A, Niemira M, Kulczynska-Przybik A. Mesenchymal stromal cells effectively limit house dust mite extract-induced mixed granulocytic lung inflammation. Allergy. 2024;79(11):3157–61. PMID: 39031017. Available from: https://doi.org/10.1111/all.16245.
50. Whitehead GS, Thomas SY, Shalaby KH, Nakano K, Moran TP, Ward JM. TNF is required for TLR ligand-mediated but not protease-mediated allergic airway inflammation. The Journal of Clinical Investigation. 2017;127(9):3313–26. PMID: 28758900. Available from: https://doi.org/10.1172/JCI90890.
51. Liu J, Chang G, Huang J, Wang Y, Ma N, Roy AC. Sodium Butyrate Inhibits the Inflammation of Lipopolysaccharide-Induced Acute Lung Injury in Mice by Regulating the Toll-Like Receptor 4/Nuclear Factor κB Signaling Pathway. Journal of Agricultural and Food Chemistry. 2019;67(6):1674–82. PMID: 30661349. Available from: https://doi.org/10.1021/acs.jafc.8b06359.
52. Liu S, Chen X, Zhang S, Wang X, Du X, Chen J, et al. miR-106b-5p targeting SIX1 inhibits TGF-$\beta$1-induced pulmonary fibrosis and epithelial-mesenchymal transition in asthma through regulation of E2F1. International Journal of Molecular Medicine. 2021;47(3):24. PMID: 33495833. Available from: https://doi.org/10.3892/ijmm.2021.4857.
53. Wheeler MJ, Green DJ, Ellis KA, Cerin E, Heinonen I, Naylor LH, et al. Distinct effects of different adjuvants in the mouse model of allergic airway inflammation. Asian Pacific Journal of Allergy and Immunology. 2020;40(2):111–120. Available from: https://doi.org/10.12932/AP-301219-0729.
54. Heldner A, Alessandrini F, Russkamp D, Heine S, Schnautz B, Chaker A. Immunological effects of adjuvanted low-dose allergoid allergen-specific immunotherapy in experimental murine house dust mite allergy. Allergy. 2022;77(3):907–19. PMID: 34287971. Available from: https://doi.org/10.1111/all.15012.
55. Shilovskiy IP, Sundukova MS, Babakhin AA, Gaisina AR, Maerle AV, Sergeev IV, et al. Experimental protocol for development of adjuvant-free murine chronic model of allergic asthma. Journal of Immunological Methods. 2019;468:10–9. PMID: 30880263. Available from: https://doi.org/10.1016/j.jim.2019.03.002.
56. Blanco O, Ramírez W, Lugones Y, Díaz E, Morejón A, Rodríguez VS, et al. Protective effects of Surfacen in allergen-induced asthma mice model. International Immunopharmacology. 2022;102:108391. PMID: 34836793. Available from: https://doi.org/10.1016/j.intimp.2021.108391.
57. Ding F, Fu Z, Liu B. Lipopolysaccharide Exposure Alleviates Asthma in Mice by Regulating Th1/Th2 and Treg/Th17 Balance. Medical Science Monitor. 2018;24:3220–9. PMID: 29768397. Available from: https://doi.org/10.12659/MSM.905202.
58. Yu QL, Chen Z. Establishment of different experimental asthma models in mice. Experimental and Therapeutic Medicine. 2018;15(3):2492–8. PMID: 29456654. Available from: https://doi.org/10.3892/etm.2018.5721.
59. Lu D, Lu J, Ji X, Ji Y, Zhang Z, Peng H, et al. IL 27 suppresses airway inflammation, hyperresponsiveness and remodeling via the STAT1 and STAT3 pathways in mice with allergic asthma. International Journal of Molecular Medicine. 2020;46(2):641–52. PMID: 32626920. Available from: https://doi.org/10.3892/ijmm.2020.4622.
60. Kondo M, Tezuka T, Ogawa H, Koyama K, Bando H, Azuma M. Lysophosphatidic Acid Regulates the Differentiation of Th2 Cells and Its Antagonist Suppresses Allergic Airway Inflammation. International Archives of Allergy and Immunology. 2021;182(1):1–13. PMID: 32846422. Available from: https://doi.org/10.1159/000509804.
61. Lee JE, Im DS. Suppressive Effect of Carnosol on Ovalbumin-Induced Allergic Asthma. Biomolecules & Therapeutics. 2021;29(1):58–63. PMID: 32632049. Available from: https:

//doi.org/10.4062/biomolther.2020.050.
62. Gong S, Ji X, Su J, Wang Y, Yan X, Wang G, et al. Yeast Fermentate Prebiotic Ameliorates Allergic Asthma, Associating with Inhibiting Inflammation and Reducing Oxidative Stress Level through Suppressing Autophagy. Mediators of Inflammation. 2021;2021:4080935. PMID: 33542675. Available from: https://doi.org/10.1155/2021/4080935.
63. Campa CC, Silva RL, Margaria JP, Pirali T, Mattos MS, Kraemer LR. Inhalation of the prodrug PI3K inhibitor CL27c improves lung function in asthma and fibrosis. Nature Communications. 2018;9(1):5232. PMID: 30542075. Available from: https://doi.org/10.1038/s41467-018-07698-6.
64. Liu Y, Chen Z, Xu K, Wang Z, Wu C, Sun Z. Next generation sequencing for miRNA profile of spleen CD4+ T cells in the murine model of acute asthma. Epigenomics. 2018;10(8):1071–83. PMID: 29737865. Available from: https://doi.org/10.2217/epi-2018-0043.
65. Huang C, Zhang Z, Wang L, Liu J, Gong X, Zhang C. ML-7 attenuates airway inflammation and remodeling via inhibiting the secretion of Th2 cytokines in mice model of asthma. Molecular Medicine Reports. 2018;17(5):6293–300. PMID: 29512725. Available from: https://doi.org/10.3892/mmr.2018.8683.
66. Khumalo J, Kirstein F, Hadebe S, Brombacher F. IL-4R$\alpha$ signaling in CD4+CD25+FoxP3+ T regulatory cells restrains airway inflammation via limiting local tissue IL-33. JCI Insight. 2020;5(20):e136206. PMID: 32931477. Available from: https://doi.org/10.1172/jci.insight.136206.
67. Mattos MS, Ferrero MR, Kraemer L, Lopes GA, Reis DC, Cassali GD, et al. CXCR1 and CXCR2 Inhibition by Ladarixin Improves Neutrophil-Dependent Airway Inflammation in Mice. Frontiers in Immunology. 2020;11:566953. PMID: 33123138. Available from: https://doi.org/10.3389/fimmu.2020.566953.
68. Safar HA, El-Hashim AZ, Amoudy H, Mustafa AS. Mycobacterium tuberculosis-Specific Antigen Rv3619c Effectively Alleviates Allergic Asthma in Mice. Frontiers in Pharmacology. 2020;11:532199. PMID: 33101014. Available from: https://doi.org/10.3389/fphar.2020.532199.
69. Camargo LD, Santos TMD, de Andrade FC, Fukuzaki S, Lopes FDDS, de Arruda Martins M. Bronchial Vascular Remodeling Is Attenuated by Anti-IL-17 in Asthmatic Responses Exacerbated by LPS. Frontiers in Pharmacology. 2020;11:1269. PMID: 33013361. Available from: https://doi.org/10.3389/fphar.2020.01269.
70. Zhu FF, Wang YM, He GZ, Chen YF, Gao YD. Different effects of acetyl-CoA carboxylase inhibitor TOFA on airway inflammation and airway resistance in a mice model of asthma. Pharmacological Reports. 2020;72(4):1011–20. PMID: 32048254. Available from: https://doi.org/10.1007/s43440-019-00027-8.
71. Sun X, Hou T, Cheung E, Iu TN, Tam VW, Chu IM, et al. Anti-inflammatory mechanisms of the novel cytokine interleukin-38 in allergic asthma. Cellular & Molecular Immunology. 2020;17(6):631–46. PMID: 31645649. Available from: https://doi.org/10.1038/s41423-019-0300-7.
72. Wang C, Liu Q, Chen F, Xu W, Zhang C, Xiao W. IL-25 Promotes Th2 Immunity Responses in Asthmatic Mice via Nuocytes Activation. PLoS One. 2016;11(9):e0162393. PMID: 27617447. Available from: https://doi.org/10.1371/journal.pone.0162393.
73. Kwak DW, Park D, Kim JH. Leukotriene B4 receptors play critical roles in house dust mites-induced neutrophilic airway inflammation and IL-17 production. Biochemical and Biophysical Research Communications. 2021;534:646–52. PMID: 33256981. Available from: https://doi.org/10.1016/j.bbrc.2020.11.027.
74. Sun Z, Ji N, Ma Q, Zhu R, Chen Z, Wang Z. Epithelial-Mesenchymal Transition in Asthma Airway Remodeling Is Regulated by the IL-33/CD146 Axis. Frontiers in Immunology. 2020;11:1598. PMID: 32793232. Available from: https://doi.org/10.3389/fimmu.2020.01598.
75. Khumalo J, Kirstein F, Scibiorek M, Hadebe S, Brombacher F. Therapeutic and prophylactic deletion of IL-4Ra-signaling ameliorates established ovalbumin induced allergic asthma. Allergy. 2020;75(6):1347–60. PMID: 31782803. Available from: https://doi.org/10.1111/all.14137.
76. Raemdonck K, Baker K, Dale N, Dubuis E, Shala F, Belvisi MG, et al. CD4+ and CD8+ T cells play a central role in a HDM driven model of allergic asthma. Respiratory Research. 2016;17(1):45. PMID: 27112462. Available from: https://doi.org/10.1186/s12931-016-0359-y.
77. Luo W, Hu J, Xu W, Dong J. Distinct spatial and temporal roles for Th1, Th2, and Th17 cells in asthma. Frontiers in Immunology. 2022;13:974066. PMID: 36032162. Available from: https://doi.org/10.3389/fimmu.2022.974066.
78. Ahuja SK, Manoharan MS, Harper NL, Jimenez F, Hobson BD, Martinez H. Preservation of epithelial cell barrier function and muted inflammation in resistance to allergic rhinoconjunctivitis from house dust mite challenge. The Journal of Allergy and Clinical Immunology. 2017;139(3):844–54. PMID: 27658763. Available from: https://doi.org/10.1016/j.jaci.2016.08.019.
79. Drake MG, Scott GD, Blum ED, Lebold KM, Nie Z, Lee JJ, et al. Eosinophils increase airway sensory nerve density in mice and in human asthma. Science Translational Medicine. 2018;10(457):eaar8477. PMID: 30185653. Available from: https://doi.org/10.1126/scitranslmed.aar8477.
80. Koopmans T, Hesse L, Nawijn MC, Kumawat K, Menzen MH, Bos IST. Smooth-muscle-derived WNT5A augments allergen-induced airway remodelling and Th2 type inflammation. Scientific Reports. 2020;10(1):6754. PMID: 32317758. Available from: https://doi.org/10.1038/s41598-020-63741-x.
81. Pan J, Yang Q, Zhou Y, Deng H, Zhu Y, Zhao D. MicroRNA-221 Modulates Airway Remodeling via the PI3K/AKT Pathway in OVA-Induced Chronic Murine Asthma. Frontiers in Cell and Developmental Biology. 2020;8:495. PMID: 32714925. Available from: https://doi.org/10.3389/fcell.2020.00495.
82. Dong L, Wang Y, Zheng T, Pu Y, Ma Y, Qi X, et al. Hypoxic hUCMSC-derived extracellular vesicles attenuate allergic airway inflammation and airway remodeling in chronic asthma mice. Stem Cell Research & Therapy. 2021;12(1):4. PMID: 33407872. Available from: https://doi.org/10.1186/s13287-020-02072-0.
83. Mazarakis N, Vongsvivut J, Bambery KR, Ververis K, Tobin MJ, Royce SG. Investigation of molecular mechanisms of experimental compounds in murine models of chronic allergic airways disease using synchrotron Fourier-transform infrared microspectroscopy. Scientific Reports. 2020;10(1):11713. PMID: 32678217. Available from: https://doi.org/10.1038/s41598-020-68671-2.
84. Akkoc T, O'Mahony L, Ferstl R, Akdis C, Akkoc T. Mouse Models of Asthma: Characteristics, Limitations and Future Perspectives on Clinical Translation. In: Turksen, K. (eds) Cell Biology and Translational Medicine, Volume 15. Advances in Experimental Medicine and Biology(), vol 1376. Springer, Cham; 2021. Available from: https://doi.org/10.1007/5584_2021_654.
85. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science. 2019;365(6452):365. PMID: 31371577. Available from: https://doi.org/10.1126/science.aaw4361.
86. Alessandrini F, Musiol S, Schneider E, Blanco-Pérez F, Albrecht M. Mimicking Antigen-Driven Asthma in Rodent Models-How Close Can We Get? Frontiers in Immunology. 2020;11:575936. PMID: 33101301. Available from: https://doi.org/10.3389/fimmu.2020.575936.
87. Chen CC, Kobayashi T, Iijima K, Hsu FC, Kita H. IL-33 dysregulates regulatory T cells and impairs established immunologic tolerance in the lungs. The Journal of Allergy and Clinical Immunology. 2017;140(5):1351–1363.e7. PMID: 28196763. Available from: https://doi.org/10.1016/j.jaci.2017.01.015.
88. Kobayashi T, Iijima K, Matsumoto K, Lama JK, Kita H. Lung-resident CD69+ST2+ TH2 cells mediate long-term type 2 memory to inhaled antigen in mice. The Journal of Allergy and Clinical Immunology. 2023;152(1):167–181.e6. PMID: 36720287. Available from: https://doi.org/10.1016/j.jaci.2023.01.016.

89. Chen JC, Chan CC, Wu CJ, Ou LS, Yu HY, Chang HL. Fetal Phagocytes Take up Allergens to Initiate T-Helper Cell Type 2 Immunity and Facilitate Allergic Airway Responses. American Journal of Respiratory and Critical Care Medicine. 2016;194(8):934–47. PMID: 27064309. Available from: https://doi.org/10.1164/rccm.201508-1703OC.
90. Bracken SJ, Adami AJ, Rafti E, Schramm CM, Matson AP. Regulation of IgE activity in inhalational tolerance via formation of IgG anti-IgE/IgE immune complexes. Clinical and Molecular Allergy : CMA. 2018;16(1):13. PMID: 29796009. Available from: https://doi.org/10.1186/s12948-018-0091-x.
91. Wang J, Zhou Y, Zhang H, Hu L, Liu J, Wang L. Pathogenesis of allergic diseases and implications for therapeutic interventions. Signal Transduction and Targeted Therapy. 2023;8(1):138. PMID: 36964157. Available from: https://doi.org/10.1038/s41392-023-01344-4.
92. Choi JY, Lee HY, Hur J, Kim KH, Kang JY, Rhee CK, et al. TRPV1 Blocking Alleviates Airway Inflammation and Remodeling in a Chronic Asthma Murine Model. Allergy, Asthma & Immunology Research. 2018;10(3):216–24. PMID: 29676068. Available from: https://doi.org/10.4168/aair.2018.10.3.216.
93. Li H, Bi Q, Cui H, Lv C, Wang M. Suppression of autophagy through JAK2/STAT3 contributes to the therapeutic action of rhynchophylline on asthma. BMC Complementary Medicine and Therapies. 2021;21(1):21. PMID: 33413331. Available from: https://doi.org/10.1186/s12906-020-03187-w.
94. Wu CT, Lin FH, Lee YT, Ku MS, Lue KH. Effect of Lactobacillus rhamnosus GG immunopathologic changes in chronic mouse asthma model. Journal of Microbiology, Immunology, and Infection = Wei Mian Yu Gan Ran Za Zhi. 2019;52(6):911–9. PMID: 30952512. Available from: https://doi.org/10.1016/j.jmii.2019.03.002.
95. Lee YT, Wu CT, Sun HL, Ko JL, Lue KH. Fungal immunomodulatory protein-fve could modulate airway remodel through by affect IL17 cytokine. Journal of Microbiology, Immunology, and Infection = Wei Mian Yu Gan Ran Za Zhi. 2018;51(5):598–607. PMID: 28709839. Available from: https://doi.org/10.1016/j.jmii.2017.06.008.
96. Vroman H, Bergen IM, van Hulst JA, van Nimwegen M, van Uden D, Schuijs MJ, et al. TNF-$\alpha$-induced protein 3 levels in lung dendritic cells instruct TH2 or TH17 cell differentiation in eosinophilic or neutrophilic asthma. The Journal of Allergy and Clinical Immunology. 2018;141(5):1620–1633.e12. PMID: 28888782. Available from: https://doi.org/10.1016/j.jaci.2017.08.012.
97. Li S, Miao Z, Tian Y, Wang H, Wang S, He T. Limethason reduces airway inflammation in a murine model of ovalbumin-induced chronic asthma without causing side effects. Experimental and Therapeutic Medicine. 2018;15(3):2269–76. PMID: 29456634. Available from: https://doi.org/10.3892/etm.2018.5691.
98. Turner DL, Goldklang M, Cvetkovski F, Paik D, Trischler J, Barahona J. Biased Generation and In Situ Activation of Lung Tissue-Resident Memory CD4 T Cells in the Pathogenesis of Allergic Asthma. The Journal of Immunology : Official Journal of the American Association of Immunologists. 2018;200(5):1561–9. PMID: 29343554. Available from: https://doi.org/10.4049/jimmunol.1700257.