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Evaluation of the differences between acute and chronic asthma models with OVA/Alum exposure

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ABSTRACT

This review article discusses the differences between animal models using ovalbumin (OVA) and aluminum hydroxide (alum). OVA, derived from chicken eggs, is a widely used allergen due to its low cost and high purity. Environmental sensitization and/or using an adjuvant such as alum is required to induce an asthma-like response. Animal species and strain, as well as sex selection, influence the development of allergic airway inflammation and other asthma-related features *in vivo* models. Acute asthma models include OVA and alum to elicit airway inflammation, elevated IgE levels, and airway hyperresponsiveness. However, these models have limitations as they do not fully mimic the chronic inflammation and airway remodeling observed in human asthma. On the other hand, chronic asthma models involve prolonged exposure to low concentrations of allergens and have been shown to exhibit persistent airway hyperresponsiveness, airway remodeling, and other critical features of asthma. The aforementioned models have provided valuable insights into the pathophysiology of asthma and have been used to evaluate potential therapeutic agents. Overall, the use of OVA and alum in animal models has improved our understanding of asthma, and it is hoped that it has the potential to guide clinical therapies in the future.

Keywords: OVA, alum, asthma, *in vivo* models, mouse

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INTRODUCTION

Ovalbumin (OVA) from chicken eggs is the most frequently used model allergen due to its low cost and ability to be obtained in the highest purity[1]. In model formation, OVA administration induces immune tolerance. To generate a comprehensive asthma-like response in OVA-based models, environmental sensitization and/or the use of an adjuvant are necessary. Animal models may also favor proteins or extracts derived from human allergens. Ragweed, extract of house dust mites (HDM) extract, and fungi, including Alternaria alternata and Aspergillus fumigatus, are examples of such allergens[2]. When allergens such as OVA induce asthmatic responses in rodents, sensitization via a systemic route, particularly intraperitoneal, is frequently required. Additionally, to enhance the immunogenicity of the sensitizing allergen, alum is used to stimulate Th2 phenotype immune responses selectively. Most related research studies indicate that OVA and alum are commonly employed either sequentially or through repeated short/long-term applications. The exploration of various lung-related pathological conditions and the advancement of novel treatments are progressing rapidly, facilitated by *in vivo* models. This review compares and assesses three mouse models according to a selection of species/strain, acute, and chronic asthma models utilized thus far.

Determination of species and strain

Animal models of experimental allergic asthma permit the identification of disease development mechanisms, the examination of disease progression over time, and the evaluation of therapeutic agents' potential to reduce the severity of inflammation in allergic asthma[3]. Different animal species can be selected depending on the subject of the research. Mice, the most commonly used species in animal models, are preferred due to their short gestation period and large number of inbred strains.

Mice are used as animal models to study many diseases, such as asthma[2]. There are sensitization and challenge phases to mimic human asthma pathogenesis in animal models. OVA challenge models are valuable tools for understanding the effects and mechanisms of asthma disease, as it is a significant allergen. These *in vivo* models provide insights into the impact of OVA and the potential therapeutic agents for asthma. The choice of mouse strain directly affects the allergic inflammation response in OVA-induced asthma models[4]. A study by Kung et al. described an allergic pulmonary inflammation model using OVA[5]. Research with mice on asthma and the Th2 high asthma phenotype has led to an improvement in the pathophysiology of asthma.

Gender is among the most fundamental characteristics that differentiate individuals from one another. Gender disparities influence the incidence, intensity, and therapeutic response to asthma, among other conditions. Melgert et al. demonstrated that in mouse models of asthma, a chronic inflammatory disease of the airways, female mice are more likely to develop allergic airway inflammation and hyperresponsiveness than male mice[6]. The study demonstrated that the levels of total cells, eosinophils, and lymphocytes were notably elevated in female mice sensitized and challenged with OVA (OVA/OVA) compared to their male mice. However, hemotoxylin-eosin staining for histological analysis revealed a notable increase in peribronchial and perivascular inflammatory cells in female mice with OVA/OVA infection compared to their male mice. When cytokine levels in the bronchoalveolar lavage (BAL) fluid of mice were evaluated, another significant parameter was elevated levels of IL-13, IL-10, TGF-β cytokines, and IgE in female OVA/OVA mice compared to their male mice[7].

Asthma models

Asthma is a significant global health concern that is progressively worsening. The majority of knowledge about allergens is derived from research, particularly that which is conducted on animal models. The immunological pathways of asthma have been clarified, and the potential of candidate therapeutic agents has been evaluated as a result of these studies. Protocol variations within and between acute and chronic asthma models impact the physiological responses obtained, as shown in (Table 1), a summary of the OVA and alum models.

Table 1. Mouse models of acute and chronic asthma models

Model	Gender/ Strain	Allergen	Sensitization	Challenge	Response to Challenge	References
Acute	Balb/c	OVA	OVA+ alum (i.p) on days 1 and 14 (20mg OVA+2 mg alum in 200ml PBS)	OVA aerosol on days 21-23 (100mg in 20mL PBS)	Inflammatory cell infiltration in bronchiole, mucus hypersecretion, alveolar congestion, alveolar wall edema	8
Acute	Balb/c	OVA	OVA+ alum (i.p) on days 0-7 (20mg OVA+2 mg alum in 200ml PBS)	OVA (i.t) on days 14-20 (0,1% OVA in 30ml PBS)	Mucus hypersecretion, goblet cell hyperplasia, peribronchial, perivascular airway epithelial thickening	9
Chronic	Balb/c	OVA	OVA + alum aerosol on days 1-12 (0,01 mg/mouse in 200mL alum)	OVA aerosol 18-23 and 26-55 (aerosolized 5% OVA)	Airway hypersensitivity, increased airway smooth muscle mass, goblet cell hyperplasia, increased number of bronchial mucosal eosinophils, peribronchiolar inflammation	10
Chronic	Balb/c	OVA	OVA + alum (i.p) on days 0-14 (10 mg OVA +2 mg alum in 100ml PBS)	OVA aerosol on days 21-24 (aerosolized 1% OVA)	Goblet cell hyperplasia bronchitis and bronchovascular inflammation eosinophil infiltration in airway epithelium and lung tissue	11

Acute asthma

Asthma does not develop spontaneously in animals compared to humans; therefore, the animal species used to study the asthma model requires the administration of external allergens[12]. It has been reported that the OVA-induced mouse asthma model observed airway hyperresponsiveness, elevated IgE levels, goblet cell hyperplasia, and airway remodeling effects[12,13]. Although many different sensitization and challenge protocols have been applied, researchers consider the basic model more consistent. If an adjuvant is present in acute sensitization protocols multiple systemic allergen administration is required. Aluminum hydroxide (alum), one of the most commonly used adjuvants, is known to stimulate a Th2-directed immune response by the immune system when exposed to an antigen[12].

Airway inflammation, goblet cell hyperplasia, elevated IgE levels, and airway hyperresponsiveness to particular allergens are fundamental asthma charac-

teristics observed in acute asthma models. However, differences can also be observed in the models that are obtained. Allergens are exposed to elevated concentrations for short periods in the models. On the contrary, long-term exposure to low concentrations of allergens induces asthma in humans. Furthermore, many of the key features of asthma appear over a short period, with some model studies showing that airway inflammation and hyperresponsiveness patterns disappear within a few weeks of the last allergen administration[14]. Although acute and chronic airway wall inflammation is characteristic of human asthma, acute, perivascular, and peribronchial inflammation of the lung parenchyma was observed in a mouse model of acute asthma[15]. The research has demonstrated that acute asthma models do not undergo alterations in airway remodeling, including subepithelial fibrosis, epithelial proliferation, and chronic inflammation of the airway[16]. Despite these noticeable limits, models of acute asthma have been effectively employed to examine the correlation between inflammatory cells and the mediating mechanisms at play, as well as the effect of the stages occurring in the lungs. The understanding of asthma as a Th2-biased disease, the function of T cells and eosinophils in the allergic response, and their impact on airway processes have all been enhanced by research[12].

Chronic asthma

The research on specific issues encountered in acute asthma models leads to examining chronic asthma models. The purpose of chronic asthma models is to accurately replicate the medical symptoms of the condition, including persistent airway hyperresponsiveness (AHR) and airway remodeling, and evaluate potential therapeutic agents. Through up to 12 weeks, the airways are exposed to low concentrations of allergens to generate the models. Several studies have identified various allergens that are known to cause allergic reactions. These include OVA[17], Lipopolysaccharide (LPS)[18], house dust mites (HDM)[19], *Alternata alternatia*[20], cockroach extracts[21], and pollen[22]. Chronic exposure to allergens has been shown to cause allergen-induced sensitization, Th2-directed allergic inflammation owing to eosinophils in the mucosa of the airways, and AHR in mice. Furthermore, certain models have demonstrated the presence of goblet cell hyperplasia, subepithelial or peribronchial fibrosis, and airway remodeling, in addition to the mentioned asthma reaction patterns. These responses are followed by airway smooth muscle thickening[23]. These patterns lead to airway remodeling asthma observations in adult humans[24]. As a result of chronic allergen exposure, some of the key features of asthma have been to persist after model application[19,25].

Mouse models of chronic asthma induced by OVA exhibited peribronchial

inflammation, dysregulation of extracellular matrix proteins, increased tissue accumulation, and subepithelial collagen deposition due to prolonged allergen stimulation. These histopathologic data are known markers of airway remodeling in a chronic asthma model. A study demonstrated an increase in the cytokines IL-4, IL-5 and IL-13 in the lung samples of mice subjected to an ELISA assay[10]. In another study indicating the chronic asthma model, it has been observed that the levels of IL-4, IL-5 and IL-1β cytokines exhibit an elevation[11]. Previous research has indicated that IL-1β, a pro-inflammatory cytokine, attracts monocytes and macrophages during the OVA sensitization and challenge stages[26]. A reduction in the quantity of regulatory T cells (Treg cells) was observed during the analysis of lymphocytes from the spleen of mice[11]. Previous studies show that asthmatic inflammation leads to the suppression of Treg cells[27]. Chronic asthma models can be used to research the basic pathological mechanisms of asthma as a result of allergen exposure. Chronic asthma models provide a suitable system for the developing new therapeutic agents for asthma.

METHODOLOGY

The focus of the search was clarified by searching the PubMed database for current mouse models of asthma. Mouse strains and allergens were identified as common components of the search. The selection was based on the application, time intervals, frequency during the sensitization and provocation phases for the acute and chronic asthma models. The different concentrations and application times of allergens were highlighted in the selected articles.

RESULTS and DISCUSSION

Asthma is a heterogeneous disease for which much of the pathophysiology is still unknown. Animal models are used to map asthma's immunological pathways and to demonstrate the efficacy of many research components involved in critical regions of these pathways. Spontaneous resolution of the response in OVA-induced acute asthma models in a short period, such as a few days, is limited in its ability to reflect the clinical features of asthma[28]. Acute asthma models only allow us to focus on the mechanisms of asthma development. The limitations of acute models pave the way for developing chronic asthma models. Our review evaluated the results of selected acute and chronic models. A model example of acute asthma was presented in the study by Wu et al. which reported the presence of thickened lung tissues and alveolar walls and infiltration of inflammatory cells[8]. A similar result was obtained in a study of airway inflammation in an OVA-induced asthma model[29]. Mucus hypersecretion in the airway epithelium is one of the most important findings in allergic asth-

ma[30]. In addition, BAL fluid cell counts revealed high concentrations of eosinophils, which have been identified as contributing to allergic inflammation[31]. Lung tissue analysis revealed high levels of the cytokines IL-4, IL-5 and IL-13[25]. OVA and alum have been shown to stimulate Th2-directed immune pathways in acute asthma models[32]. Histopathological evaluation of the acute asthma model studied by Rajasekar et al. showed the presence of epithelial thickening, goblet cell hyperplasia, and mucus hypersecretion in the peribronchial and perivascular airways compared to the control group. The number of eosinophils, neutrophils and macrophages in the BAL fluid increased with OVA exposure. Increased levels of IgE, a known marker of airway inflammation, and IL-4, a member of the Th2 cytokine family, were observed. In addition, levels of TNF-α and IFN-γ, both pro-inflammatory cytokines, were elevated compared with the control group[9]. There were differences between the two models of acute asthma in the timing of OVA and alum administration during the sensitization phase and the days and route of OVA administration during the challenge phase. Despite these differences, histopathological markers of airway inflammation were obtained in both models, and Th2 cytokine-driven immunity was shown to occur with the cytokines analyzed from BAL fluid[8,9]. Acute asthma models are helpful for studying the pathophysiological patterns that occur in the acute phase of asthma. However, chronic asthma models are preferred to study the pathways involved in the chronic asthma process and the effect on airway remodeling. The two studies reviewed used comparable chronic asthma models[10,11]. The doses and duration of administration varied in the models. Despite the different doses and durations, common asthma phenotypes such as goblet hyperplasia, inflammation of BAL cells, and increase in Th2 cytokines, AHR, and eosinophil filtration were observed in mouse models in both studies[10,11]. In chronic asthma mouse models, the duration and dose of protocol modifications depend on the specific aspect of asthma being studied. Still, the common allergens used provide similar results in asthma pathogenesis. It has also been shown that repeated allergen challenges in cricket asthma models can mimic critical markers of human asthma.

Collectively, mouse models are often the preferred method to study asthma. The complex biochemical nature of the disease makes it difficult for mouse models to mimic human asthma. The phenotypes of mouse models of chronic asthma that differ from human asthma include:

late-phase bronchoconstriction,

a different distribution of lung inflammation than in humans,

tolerance after repeated allergen exposure[1].

Therefore, model studies have focused on specific patterns of asthma rather than a holistic approach to the disease. Since many primary phenotypes seen in asthma can be reproduced in OVA models, it was a suitable model choice. Although no mouse model completely mimics asthmatic patients' pathology and physiology, animal models using OVA and alum have accelerated the understanding of the biochemical mechanisms underlying asthma and the investigation of therapeutic targets. The timing of OVA and alum administration in animal models varies depending on the specific characteristics of asthma to be studied. Variations in model protocols lead to differences in the results obtained. In this way, asthma can be investigated in more detail through the specific mechanism of action established. This review will help researchers choose the more appropriate asthma models among differential asthma patterns for their studies. The review is expected to contribute to more detailed modeling of asthma disease by enabling essential science findings from model systems to help pre-clinical studies.

STATEMENT OF ETHICS

Ethical approval does not apply to this article.

CONFLICT OF INTEREST STATEMENT

The author declares that there are no conflicts of interest regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

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