



Mouse Models of Asthma: Characteristics, Limitations and Future Perspectives on Clinical Translation

Tolga Akkoc, Liam O'Mahony, Ruth Ferstl, Cezmi Akdis, and Tunc Akkoc

Abstract

Asthma is a complex and heterogeneous inflammatory airway disease primarily characterized by airway obstruction, which affects up to 15% of the population in Westernized countries with an increasing prevalence. Descriptive laboratory and clinical studies reveal that allergic asthma is due to an immunological inflammatory response and is significantly influenced by an individual's genetic background and environmental factors. Due to the limitations associated with human experiments and tissue isolation, direct

mouse models of asthma provide important insights into the disease pathogenesis and in the discovery of novel therapeutics. A wide range of asthma models are currently available, and the correct model system for a given experimental question needs to be carefully chosen. Despite recent advances in the complexity of murine asthma models, for example humanized murine models and the use of clinically relevant allergens, the limitations of the murine system should always be acknowledged, and it remains to be seen if any single murine model can accurately replicate all the clinical features associated with human asthmatic disease.

T. Akkoc (✉)

Genetic Engineering and Biotechnology Institute, Tubitak Marmara Research Center, Kocaeli, Turkey
e-mail: tolga.akkoc@tubitak.gov.tr

L. O'Mahony

Department of Medicine and Microbiology, APC Microbiome Ireland, University College Cork, Cork, Ireland

R. Ferstl

Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

C. Akdis

Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

T. Akkoc

Department of Pediatric Allergy-Immunology, School of Medicine, Marmara University, Istanbul, Turkey

Keywords

Allergy · Asthma · Mouse models

Abbreviations

OVA	Ovalbumin
Der p	Dermatophagoides pteronyssinus
Der f	Dermatophagoides farinae
HDM	House dust mite
SPF	Specific pathogen free
ASM	airway smooth muscle
PBMCs	peripheral blood mononuclear cells

1 Introduction

Asthma is one of the most important chronic airway inflammatory diseases that affects almost 300 million people worldwide. The components of the disease are characterized by airway inflammation, variable and recurring symptoms, airway hyper-responsiveness, and chronic airflow limitations that associate with structural changes (Dharmage et al. 2019). Asthma is referred to as a complex disease where one's genetics and environmental influences play a critical role. Although evidence from twin and family studies point to a strong correlation with genetic background, the interaction between genetic and environmental factors is still under investigation (Pivniouk et al. 2020). From a clinical perspective, asthma shows differences in severity, natural history comorbidities, and medication/treatment response. Although it is difficult to strictly categorize into defined groups, the Tucson study proposed three classical phenotypes of childhood wheezing: transient early wheezers, nonatopic wheezers, and persistent atopic wheezers (Taussig et al. 1989). The term "endotype" has been proposed to define an asthma subtype characterized by a distinct pathophysiological mechanism (Akar-Ghibril et al. 2020). Recent studies revealed that parameters such as clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and treatment response are useful in the determination of asthma sendotypes (Crescitelli et al. 2021). Currently proposed asthma endotypes include eosinophilic asthma, exacerbation-prone asthma, exercise-induced asthma, adult-onset asthma, fixed airflow limitation, and poorly steroid-responsive asthma. Among these subphenotypes, the current murine models only represent early onset Th2-type allergic asthma and eosinophilic asthma (Periz et al. 2020).

From the immunological perspective, allergic asthma is defined as a Th2-type disease state. A number of studies have demonstrated increased eosinophils and Th2-derived cytokines in the airways of children with severe asthma (Caspard et al. 2020; Cevhertas et al. 2020), while others

have noted noneosinophilic patterns of airway inflammation with increased neutrophils and biomarkers of neutrophil activation (Hauk et al. 2008; Lex et al. 2005; Moeller et al. 2015). Thus, severe asthma can be associated with different endotypes that do not represent a Th1 or Th2 cytokine pattern (Fitzpatrick et al. 2010; Steinke et al. 2021).

The correct animal model should reflect the disease pathophysiology as closely as possible and is essential for the development of new therapies. Because of ethical concerns, clinical studies with allergic individuals are limited in scope and experimental murine models have become more important. Although, today there are several therapeutic and preventive animal models for allergic asthma, there remain important components to be elucidated. Data from clinical specimens suggest that some features of human asthma can be replicated in animal models, thereby providing data on disease characteristics (Stevenson and Birrell 2010). Several studies carried out in animal models have given important clues that explain the pathophysiological conditions related to the disease status. For instance, the role of Th2-type cytokines in the pathogenesis of asthma have been particularly well-studied in animal models of allergic lung inflammation.

Mouse models have been developed for almost all types of allergic disease, such as asthma (Nials and Uddin 2008; Aun et al. 2017), allergic rhinitis (Wagner and Harkema 2007), food allergy and anaphylaxis (Dearman and Kimber 2007), atopic dermatitis (Jin et al. 2009), and allergic conjunctivitis (Niederkorn 2008). These models are important to examine the mechanism of the disease and the activity of a variety of genes and cellular pathways, predict the safety of new drugs or chemicals before being used in clinical studies (Karol 1994), define the pathogenic pathways, and suggest new therapeutic options (Bice et al. 2000). Although murine models of asthma provide important insights into the disease mechanisms, there are some limitations that should be considered. Beside the genetic and physiological differences between humans and

mice, there are also limitations due to complexity of this disease. In other words, mice do not develop asthma. One can replicate important components of the disease, but no single model accurately models all the features of asthma (Wenzel and Holgate 2006; Kumar and Foster 2002; Ray et al. 2015). This is very important to take into account when choosing the correct model to address the specific experimental question. For example, chronic exposure models are required to examine many of the structural changes associated with inflammatory disease within the airways. This review focuses on the different mouse models of asthma that are currently in use and discusses the limitations of each model.

1.1 Immune Response to Allergens

If immune tolerance is overcome to certain allergens, such as aeroallergens, food allergens, and insect venom, type I and type IV hypersensitivity reactions ensue. Several factors, including genetic susceptibility, the nature of antigen that initiates the disease (antigen dose, time of exposure, route of exposure, and structural characteristics) and exposure to infections and bacteria (Burks et al. 2008), influence the type of immune response.

Specialized antigen-presenting cells, such as dendritic cells, have a crucial role in the presentation of antigenic compounds to T lymphocytes and lead to differentiation of naive T cells into Th1, Th2, Th9, Th17, and Th22 effector T-cell subsets (Goldberg and Rock 1992; Akdis and Akdis 2009). In murine models, the initial event responsible for the development of allergic disease is the generation of allergen-specific CD4⁺Th2 cells (Jutel and Akdis 2011). The current view is that IL-4 stimulation promotes naive T cell differentiation into Th2 cells (Romagnani 1994; Mosmann and Sad 1996; Abdelaziz et al. 2020). The allergic immune response occurs in two main phases: first sensitization and development of memory, and later followed by the effector phase and tissue injury. In the sensitization phase, CD4⁺Th2 cells secrete IL-4, IL-5, and

IL-13 and mediate several functions. These cytokines induce class switching of antibody isotypes to the ϵ heavy chain for IgE antibody production by B cells, development and recruitment of eosinophils, production of mucus, and reduction the threshold for contraction of smooth muscle cells (Mosmann and Sad 1996; Romagnani 1994; Corry 1999). Later, this allergen-specific IgE binds to high-affinity IgE receptors (Fc ϵ RI receptors) on the surface of mast cells and basophils. This series of activation steps leads to the sensitization of patients to a specific allergen. In the final phase, reexposure to the sensitized allergens leads to activation of effector cells and tissue injury occurs. The degranulation of basophils and mast cells by IgE-mediated cross-linking of receptors is crucial in the type-I hypersensitivity reaction, which may lead to development of type-IV hypersensitivity chronic allergic inflammation. All these events require allergen-specific T cell activation in allergic individuals, while for healthy individuals peripheral, T cell tolerance prevents formation of allergic immunopathology (Hirose et al. 2017).

1.2 Mouse Models of Asthma

Mice are the most common species studied for animal models of asthma (Sagar et al. 2015). In particular, acute or chronic allergic lung inflammation models utilizing ovalbumin (OVA) or house dust mite represent severe, persistent asthma models that have been successfully established in mice. Usually, mice are systemically sensitized to allergen with alum as an adjuvant via intraperitoneal injection and allergen challenged via the airways. The number of sensitizations and challenges is decisive for the development of acute or chronic forms of this model. The nature of the lung inflammation is directly influenced by the genetic background of the mice, the allergen, type of the sensitization and challenge protocol, and contamination of the allergen with substances (e.g. LPS), which stimulate the innate immune response (Zosky and Sly 2007) (Table 1).

Table 1 Mouse models of acute and chronic asthma

Mouse strain	Sensitization	Challenge	Type of Asthma model	References
BALB/c	i.p. OVA / alum	OVA (aerosol)	Acute model	Lloyd (2007), Tomkinson et al. (2001), Hamelmann et al. (1999)
A/J	i.p. Bla g 2 and Derf 1	Allergen (oro-tracheal)	Acute model	Sarpong et al. (2003)
C57BL/6	i.p. Der p 1 / alum	HDM (aerosol)	Acute model	Tourmoy et al. (2000)
BALB/c	i.p. Bet v 1 / alum	Bet v 1 (aerosol)	Acute model	Wiedermann et al. (1999a), Winkler et al. (2002)
BALB/c	i.p. OVA / alum	OVA (aerosol, 6–8 weeks)	Chronic model	Temelkovski et al. (1998)
BALB/c	i.p. OVA / alum	OVA (i.n., 10–12 weeks)	Chronic model	Henderson et al. (2002), Leigh et al. (2002)
BALB/c	-	HDM (i.n., 5 weeks)	Chronic model	Johnson et al. (2004), Ulrich et al. (2008)
BALB/c	i.p. OVA / alum	OVA (i.t.)	Chronic model	Akkoc et al. (2001)
C.B17 SCID	i.p. PBMCs	Dpt (aerosol)	Acute model	Duez et al. (1996), Duez et al. (2000)
NOD/SCID	i.p. PBMCs	HDM (aerosol)	Acute model	Perros et al. (2009)
SCID	i.p. PBMCs, Dpt/alum	Dpt (aerosol)	Acute model	Sonar et al. (2010)
C.B17 SCID	i.p. PBMCs, i.p. Der p1	Der p1 (aerosol)	Acute model	Herz et al. (2004a)
BALB/c, C57BL/6	s.c. OVA	OVA (aerosol)	Acute model	Conrad et al. (2009)
BALB/c	i.n./i.p. OVA	OVA (i.n.)	Acute model	Nelde et al. (2001)
BALB/c	Pulsed DCs i.t.	OVA (aerosol)	Acute model	Hammad et al. (2004)
BALB/c	OVA (aerosol)	OVA (aerosol)	Acute model	Renz et al. (1992)
BALB/c	i.t. OVA	OVA (aerosol)	Acute model	Blyth et al. (1996)
BALB/c	i.n. OVA	OVA (aersol)	Acute model	Williams and Galli (2000)
BALB/c	i.p. OVA	OVA (aerosol)	Acute model	Hellings and Ceuppens (2004), Mojtabavi et al. (2002)

1.2.1 Genetic Background of Mice

Various inbred mouse strains are available in laboratories (Song and Hwang 2017). However, it is not possible to develop an asthma model with all of these mouse strains. Based on the level of allergen-specific IgE and IgG1 production and the degree of airway inflammation following repeated allergen challenges, high- and low-responder mouse strains have been identified. Each different mouse strain shows a different pattern of response following immunization to allergens. There are substantial differences in the ability to induce allergic inflammation and AHR within these mouse strains. A/J and AKR/J mice display high levels of allergen-induced AHR and reactivity to methacholine

(Ewart et al. 2000), while C3H/HeJ and DBA/2 mice are resistant to the development of allergen-induced AHR (McIntire et al. 2001).

In many studies, either BALB/c or C57BL/6 mice were used. BALB/c mice are known as IgE-high responders to many allergens (e.g. OVA, Bet v 1) and goat anti-mouse IgD-stimulation, whereas C57BL/6 mice are characterized by low-IgE response (Herz et al. 2004b; Bousquet et al. 2000; McMillan and Lloyd 2004; Van Hove et al. 2009; Birrell et al. 2003). In contrast, C57BL/6 mice exhibit relatively more Th1-dominant immune responses compared to BALB/c mice and are utilized more often as colitis models (Van Hove et al. 2009) (Melgar et al. 2005).

1.2.2 Allergens

In mouse models of asthma, different types of allergens have been used. Among them, OVA derived from chicken egg is a frequently used allergen that induces robust, allergic pulmonary inflammation in mice (Kumar et al. 2008). There are some advantages to using OVA in models, such as the low cost of materials, high purity, the immunodominant epitopes have been well characterized and synthetic peptides have been generated (Fuchs and Braun 2008). In addition, OVA-specific transgenic mice allow for the detailed characterization of T cell-dependent immune processes that lead to allergic sensitization and inflammation (Akar-Ghbiril et al. 2020).

The method of allergen administration is important for the induction of allergic asthma, while circumventing development of tolerance. For example, repeated inhalation or oral administration of OVA induces tolerance instead of sensitization (Burks et al. 2008). For the induction of allergen sensitization, the allergen is traditionally combined with an adjuvant and administered via intraperitoneal injection. However, sensitization to OVA can also be achieved without adjuvant if injected subcutaneously (Conrad et al. 2009). Following sensitization, a series of inhaled or intranasal challenges is administered to elicit an allergic inflammatory response. OVA-induced allergic airway models may not represent the same conditions experienced by asthmatic humans, who may have more frequent and much longer periods of allergen exposure (Kumar et al. 2008).

In addition, OVA is not a relevant allergen associated with human asthma. During the last five years, a great deal of work has been performed, which has led to the development of atopy models using allergens other than OVA. For this purpose, alternative allergens that have clinical relevance, such as house dust mite (HDM) allergens (i.e. *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), have been used (Cates et al. 2007; Johnson et al. 2004; Sarpong et al. 2003). Compared to the OVA model, the HDM allergy model has a number of advantages. The most

important advantage is that many asthmatic subjects have increased levels of HDM-specific IgE, and these people display exacerbated allergic events with HDM allergen (Maunsell et al. 1968; McAllen et al. 1970). In addition, experimental data revealed that HDM can sensitize animals via the respiratory mucosa and does not require adjuvant co-administration (Jarnicki et al. 2001). Finally, long-term application of HDM leads to chronic lung inflammation and associated airway remodeling, which resembles some additional features of human asthma (Johnson et al. 2004; Ulrich et al. 2008).

Furthermore, there are other disease-relevant aeroallergens, such as fungal allergens (e.g. *Aspergillus fumigatus*), ragweed, and grass pollen spores, which are being used for murine atopy models (Fuchs and Braun 2008; Wiedermann et al. 1999b; Winkler et al. 2002; Hirahara et al. 1998).

1.2.3 Sensitization and Challenge Protocols

Specific pathogen free (SPF) mice do not spontaneously develop AHR or allergic airway inflammation. Many different sensitization and challenge protocols have been compared (McMillan et al. 2002; Hessel et al. 1995; Tournoy et al. 2000; Tomkinson et al. 2001). Acute or chronic asthma models have been developed which are dependent on dose and timing of sensitization and challenge. Acute sensitization protocols usually require multiple systemic administrations of allergen, and most protocols use a combination of allergen with an adjuvant. Aluminium hydroxide (AlOH₃, Alum) is one of the preferred adjuvants that promotes the development of the allergen-specific Th2 response (Flach et al. 2011; Jensen-Jarolim et al. 2021). Recent studies suggest that alum induces uric acid secretion, which is required for the alum-associated Th2 adjuvant effects. However, alum also activates the inflammasome-IL-1 receptor pathway, and the role of this pathway in alum-induced asthma is controversial (Kool et al. 2011). As discussed above, adjuvant-free protocols are also available, which usually require

a higher number of exposures through different routes to achieve suitable sensitization (Blyth et al. 1996). Both OVA and HDM can be used as antigen to induce pulmonary inflammation. Inhaled HDM is more successful in developing an respiratory allergy model because of the intrinsic enzymatic activity associated with the contents of this allergen (Bush et al. 2011). Extracts or purified major allergens of potent human allergens, including cockroach, ragweed, or fungi, have been increasingly used as allergens in mice and other species (Barrett et al. 2003; Kurup et al. 1997; Chapoval et al. 2002; Matsuda et al. 2021). After a sensitization period (usually 14–21 days), allergen challenge via airways is carried out for several days. The administration of allergen through airways can be applied by nebulization, intratracheal (i.t), or intranasal (i.n.) instillation. With these sensitization and challenge protocols, mice develop certain features of clinical asthma, which can be defined as increased levels of allergen-specific and total IgE, eosinophil-dominant inflammatory cell infiltration to the peri-bronchial area, goblet cell hyperplasia, basement membrane thickening, and smooth muscle cell hypertrophy and AHR to specific allergens or methacholine (Takeda and Gelfand 2009).

Although an acute allergen challenge model displays many key aspects of human asthma, there are obviously limitations when one compares these models with asthmatic patients. To overcome these limitations, several research groups have developed chronic allergen challenge models in order to reproduce more of the features associated with asthma, such as goblet cell metaplasia, epithelial hypertrophy, subepithelial fibrosis, and smooth muscle hypertrophy, which are altogether referred to as airway remodeling (Akkoc et al. 2001; Blyth et al. 1996). In order to develop a chronic asthma model, low levels of allergen should be repeatedly administered to the airways for periods up to 12 weeks. In most of the experimental models, OVA has been used as an allergen (Fernandez-Rodriguez et al. 2008; Temelkovski et al. 1998; Wegmann 2008; Akkoc and Genc 2020), and in some experimental models, environmentally

relevant antigens such as HDM extract or grass pollen have been used to develop a chronic asthma model (Fuchs and Braun 2008; Maunsell et al. 1968).

1.3 Similarities and Differences Between Murine Models and Human Asthma

Mice do not exhibit asthma-like lung inflammation, which is point-by-point comparable to human asthma. There are important limitations that should be considered. Mice develop an allergen-specific Th2-type immune response using a highly artificial peritoneal allergen sensitization procedure in the presence of an adjuvant, such as alum (Debeuf et al. 2016). They do not develop spontaneous immediate and late phase airway obstruction as is seen in human asthma (Kumar and Foster 2002).

There are significant differences in the developmental features of human and mouse lungs at birth. While the human lung continues to develop from months to years after birth, mouse lungs are already mature at birth (Lodrup Carlsen and Carlsen 2001). In humans, the immature lung is exposed to environmental antigens or agents, and the immune response within the immature human lung is different compared to that of a mouse lung (Greenough et al. 2004). From the histopathological view, there are also differences in the lungs of human and mice, and this further affects the histopathological changes of chronic asthma models (Persson et al. 1997; Kumar and Foster 2002). Although submucosal glands are abundant throughout the medium and large airways of the human lung, they are only present in the trachea of mice. In the large airways of the mouse, the bronchial epithelium is not as fully stratified as in humans. This results in differences in the immune response to inhaled antigens (Lilly et al. 2005). Human asthma is restricted to conducting airways, while in mice, parenchymal and vascular components of the airways are more affected (Kumar and Foster 2002).

The role of airway smooth muscle (ASM) and myofibroblasts in the bronchi in bronchial

hyperreactivity is still under exploration (Hallstrand et al. 2011; Collins et al. 2003). In asthma, increased ASM mass is the result of hyperplasia, hypertrophy, and migration of ASM and forms one of the most important components of airway remodeling (Hirst et al. 2004; Broide 2008). ASM also contributes to the inflammatory process by producing active mediators, modifying the extracellular matrix composition, and interacting with inflammatory cells (Camoretti-Mercado 2009).

Reversible and variable airflow limitations are also key features of asthma. The most characteristic feature of asthma is the variability or periodicity of lung dysfunction, which distinguishes asthma from chronic obstructive pulmonary disease (Irvin 1987). Airway obstruction is the key feature of the disease, which is an important diagnostic criterion in the clinic. Spirometry is the easiest and most clinically useful method for measuring airflow limitation in most clinical situations (Burrows 1980). Preclinical studies for measuring respiratory function in vivo include noninvasive and invasive technologies. Both techniques have their advantages and disadvantages, and it depends on the aim of the study as to which method should be chosen (Table 2). There are two different measurements; tidal midexpiratory flow (EF₅₀) provides data on the midpoint (50%) of expiratory tidal volume in mL/s, while enhanced pause (Penh) is widely

used in order to demonstrate airway obstruction in mouse models. However, strictly speaking, Penh is a better measure of distress rather than airway obstruction (Petak et al. 2001; Bates et al. 2004; Adler et al. 2004).

Critical histopathological changes lead to increased bronchial hyperreactivity. In humans, chronic airway changes, which occur over a long period of time, lead to the severe asthma phenotype, which is observed in the clinic. In contrast, due to the shorter-term experimental period and the nature of the animal model, mice do not exhibit many of the lesions that typify chronic human asthma. In particular, there is minimal chronic inflammatory changes of the airway wall and airway remodeling, such as epithelial fibrosis, epithelial proliferation, and large increase in airway smooth muscle cells in mouse (Holgate et al. 2000). However, murine models are very useful in examining the early molecular events, which may contribute to airway remodeling, such as increased Claudin-1 expression by ASM (Fujita et al. 2011).

1.4 Cytokines: From Mouse Models to Human Asthma

The entrance of an allergen into lymphatic organs initiates a series of immunologic cascades from presentation to CD4⁺T cells by dendritic cells and

Table 2 Invasive and noninvasive techniques to measure lung function in mice

	Pros and Cons	Methods	References
Noninvasive techniques	Conscious mice Repeatable screening of respiratory function Large numbers of animals	Head out plethysmography (Tidal midexpiratory flow-EF ₅₀)	Vijayaraghavan et al. (1993), Glaab et al. (2002)
	Natural breathing pattern Simple handling Stress for animals Only volume and flow measurable; inhalation exposure includes nasal and gastrointestinal uptake	Barometric plethysmography (enhanced pause-Penh) (whole body plethysmography)	Hamelmann et al. (1997), Finkelman (2008)
Invasive techniques	Unstrained mice Anesthetized animal Repetitive/nonrepetitive No stress for animals	Orotracheally intubated animals (repetitive) (EF ₅₀)	Glaab et al. (2004), Ruckert et al. (2005)
	Gold standard parameters Inhalation exposure focus to the lungs Not very easy handling	Tracheostomized animals (nonrepetitive) (EF ₅₀)	Palecek et al. (1967)

induction of sensitized Th2 cells, which further releases IL-4, IL-5, IL-9, and IL-13 (Hamid and Tulic 2009). These cytokines play a role in airway eosinophilia, pulmonary lymphocytosis, and mastocytosis; goblet cell hyperplasia with epithelial cell proliferation; smooth muscle hyperplasia and increased thickness; subepithelial fibrosis; isotype switching of IgE and release from B cells; increased production of chemokines, which attracts T cells, eosinophils, neutrophils, and mast cells; and narrowing of the airways because of smooth muscle contraction (Hamid and Tulic 2009; Holgate 2008; Akdis et al. 2011).

Although allergy is correlated with Th2-type responses, some studies revealed that there is also a Th1 immune response in the lung of children with asthma (Brown et al. 2003). It is not unusual to see increased IFN- γ and IL-12 levels in human asthma (Kenyon et al. 2000). Similarly, diseases which have been characterized as Th1-dominant disorders can also display features normally associated with Th2-type immune responses. For instance, although Sarcoidosis is a Th1 disease, increased levels of IL-13 are also seen, which suggests a Th2 component to the disease (Hauber et al. 2003; Akdis et al. 2011; Meyer et al. 2010). One possibility is that Th2 responses are required at the initiation phase of asthma, while the inflammatory response, which occurs during the exacerbation phase, is not limited to Th2 responses but also requires Th1 and perhaps Th17 responses within the lung.

IL-4 and IL-13 are prototypical Th2-type cytokines, and several studies have revealed that they play a crucial role in the induction of murine asthma. Induction of IL-4 and IL-13 via both the intranasal and intratracheal routes enhances allergic airway disease (Venkayya et al. 2002). Enhanced transgenic expression of IL-4 in the airway epithelial Clara cells elicits an inflammatory response characterized by epithelial cell hypertrophy, with the accumulation of macrophages, lymphocytes, eosinophils, and neutrophils without any airway hyperreactivity or goblet cell hyperplasia (Rankin et al. 1996; Zhu et al. 1999). Selective pulmonary expression of IL-13 promotes a eosinophilic inflammatory response, mucus cell metaplasia, airway

obstruction, and nonspecific airway hyperreactivity (Zhu et al. 1999). In mice, IL-13 binds to its receptor, IL-13R α 2, either in soluble form in serum or membrane-bound receptor, which is expressed by smooth muscle, while human IL-13 binds only to the membrane isoform (Chen et al. 2009). Thus, murine models have been very useful in the examination of the roles played by specific cytokines in the initiation of allergic-type pulmonary inflammation due to the availability of knock-out and transgenic models. However, murine studies should be complemented by findings in humans, which confirm the relevance of the cytokine data.

1.5 Humanized Animal Models of Asthma

A humanized mouse refers to a mouse which contains functional human genes, cells, tissues, and/or organs. For this purpose, immunodeficient mice are used to receive human cells, because they can easily accept heterologous cells due to deficiencies in the host immune system. Severe combined immunodeficient (SCID) mice were demonstrated to be a useful model to analyze human pathogenic mechanisms (Alessandrini et al. 2020). Due to an immune defect, these mice are unable to reject allogenic or xenogeneic transplants (Mosier et al. 1988; McCune 1991) and show an absence of mature and functional T and B lymphocytes (Bosma et al. 1983). It has been shown that SCID mice reconstituted with human peripheral blood mononuclear cells (PBMCs) from asthmatic patients developed a specific human IgE response, pulmonary inflammatory-type infiltrate, human Th2 cytokine production, lung inflammation, and AHR when exposed to specific allergens by inhalation (Duez et al. 1996; Pestel et al. 1994; Chiang et al. 1995; Gagnon et al. 1995; Steinsvik et al. 1999). Thus, it is possible to reconstitute components of the human immune system in mice by transferring PBMCs resulting in a human–mouse chimera with a functional human antigen-reactive immune system (Herz et al. 1998; Duez et al. 2000). The newer generation of humanized mouse models

use SCID mice crossed to IL-2Receptor gamma Knockout mice, so-called NOD or NSG mice (NOD.Cg-PrkdcSCIDIL2rgtm1WjI/SzJ), which are the more efficient immunocompromised strain (Shultz et al. 2007). These mice lack mature T, B, and NK cells. In addition, they are deficient in multiple cytokine signaling pathways as well as displaying many defects in the innate immune system (Shultz et al. 1995, 2005). Since 2000, the newest generation of immunodeficient mice is available, called NOG mice (NOD/Shi-scid/IL-2R γ null). These mice accept heterologous cells more easily than NOD/scid mice. Thus, they represent a good humanized model as they are the most efficient recipients of human cells, resulting in successful engraftment, proliferation, and differentiation (Ito et al. 2002). The use of these animals in asthma research and preclinical drug development is an exciting development and should improve the identification of novel therapeutic compounds which will be useful in the clinic.

2 Conclusion

Recent studies clearly show that asthma is a complex syndrome, likely made up of a number of disease endotypes, each with distinct pathophysiology and possibly different underlying immunological mechanisms. Due to the diverse nature and complexity of asthma, it is difficult to represent all the features of asthma endotypes in murine models. Within those endotypes, the current murine models may only represent early onset Th2 and eosinophilic asthma.

Additional limitations of murine models, due to the anatomical and immunological differences between humans and mice, suggest that investigators should be careful in directly extrapolating data from murine studies to human disease. However, murine and human studies both support the importance of the initial Th2 response in the promotion of airway disease and the subsequent mixed Th1/Th2 response in sustaining inflammatory responses within the lung. Human clinical studies remain the gold standard for direct evidence for the clinical efficacy of new

therapeutic approaches. Murine models will continue to provide important mechanistic clues, while improved mouse models may be useful for extending our understanding of the basic mechanisms underpinning asthma and for examining new therapeutic options.

References

- Abdelaziz MH, Abdelwahab SF, Wan J, Cai W, Huixuan W, Jianjun C, Kumar KD, Vasudevan A, Sadek A, Su Z, Wang S, Xu H (2020) Alternatively activated macrophages; a double-edged sword in allergic asthma. *J Transl Med* 18(1):58. <https://doi.org/10.1186/s12967-020-02251-w>
- Adler A, Cieslewicz G, Irvin CG (2004) Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 97(1):286–292. <https://doi.org/10.1152/japplphysiol.00821.2003;00821.2003> [pii]
- Akar-Ghibril N, Casale T, Custovic A, Phipatanakul W (2020) Allergic Endotypes and phenotypes of asthma. *J Allergy Clin Immunol Pract* 8(2):429–440. <https://doi.org/10.1016/j.jaip.2019.11.008>
- Akdis CA, Akdis M (2009) Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol* 123(4):735–746. <https://doi.org/10.1016/j.jaci.2009.02.030>; quiz 747–738 S0091-6749(09)00377-7 [pii]
- Akdis M, Burgler S, Cramer R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O, Rhyner C, Quaked N, Schaffartzik A, Van De Veen W, Zeller S, Zimmermann M, Akdis CA (2011) Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 127(3):701–721 e701-770. <https://doi.org/10.1016/j.jaci.2010.11.050>; S0091-6749(10)01949-4 [pii]
- Akkoc T, Genc D (2020) Asthma immunotherapy and treatment approaches with mesenchymal stem cells. *Immunotherapy* 12(9):665–674. <https://doi.org/10.2217/imt-2019-0194>
- Akkoc T, Tolunay S, Barlan I, Basaran M (2001) Airway remodeling and serum total immunoglobulin E (IgE) levels in a murine model of asthma. *J Asthma* 38(7): 585–591
- Alessandrini F, Musiol S, Schneider E, Blanco-Perez F, Albrecht M (2020) Mimicking Antigen-Driven Asthma in Rodent Models-How Close Can We Get? *Front Immunol* 11:575936. <https://doi.org/10.3389/fimmu.2020.575936>
- Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P (2017) Animal models of asthma: utility and limitations. *J Asthma Allergy* 10:293–301. <https://doi.org/10.2147/JAA.S121092>

- Barrett EG, Rudolph K, Bowen LE, Muggenburg BA, Bice DE (2003) Effect of inhaled ultrafine carbon particles on the allergic airway response in ragweed-sensitized dogs. *Inhal Toxicol* 15(2):151–165. <https://doi.org/10.1080/089583703044474>; 3KNN5GM2CNUB99LW [pii]
- Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, Eidelman D, Ludwig M, Macklem P, Martin J, Milic-Emili J, Hantos Z, Hyatt R, Lai-Fook S, Leff A, Solway J, Lutchen K, Suki B, Mitzner W, Pare P, Pride N, Sly P (2004) The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 31(3):373–374; 31/3/373 [pii]
- Bice DE, Seagrave J, Green FH (2000) Animal models of asthma: potential usefulness for studying health effects of inhaled particles. *Inhal Toxicol* 12(9):829–862. <https://doi.org/10.1080/08958370050123207>
- Birrell MA, Battram CH, Woodman P, McCluskie K, Belvisi MG (2003) Dissociation by steroids of eosinophilic inflammation from airway hyperresponsiveness in murine airways. *Respir Res* 4:3
- Blyth DI, Pedrick MS, Savage TJ, Hessel EM, Fattah D (1996) Lung inflammation and epithelial changes in a murine model of atopic asthma. *Am J Respir Cell Mol Biol* 14(5):425–438
- Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. *Nature* 301(5900):527–530
- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM (2000) Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 161(5):1720–1745
- Broide DH (2008) Immunologic and inflammatory mechanisms that drive asthma progression to remodeling. *J Allergy Clin Immunol* 121(3):560–570. <https://doi.org/10.1016/j.jaci.2008.01.031>; quiz 571–562. S0091-6749(08)00231-5 [pii]
- Brown V, Warke TJ, Shields MD, Ennis M (2003) T cell cytokine profiles in childhood asthma. *Thorax* 58(4):311–316
- Burks AW, Laubach S, Jones SM (2008) Oral tolerance, food allergy, and immunotherapy: implications for future treatment. *J Allergy Clin Immunol* 121(6):1344–1350. <https://doi.org/10.1016/j.jaci.2008.02.037>; S0091-6749(08)00424-7 [pii]
- Burrows B (1980) Course and prognosis of patients with chronic airways obstruction. *Chest* 77(2 Suppl): 250–251
- Bush RK, Swenson C, Fahlberg B, Evans MD, Esch R, Busse WW (2011) House dust mite sublingual immunotherapy: results of a US trial. *J Allergy Clin Immunol* 127(4):974–981 e971–977. <https://doi.org/10.1016/j.jaci.2010.11.045>; S0091-6749(10)01939-1 [pii]
- Camoretti-Mercado B (2009) Targeting the airway smooth muscle for asthma treatment. *Transl Res* 154(4):165–174. <https://doi.org/10.1016/j.trsl.2009.06.008>; S1931-5244(09)00184-4 [pii]
- Caspard H, Ambrose CS, Tran TN, Chipps BE, Zeiger RS (2020) Associations between individual characteristics and blood eosinophil counts in adults with asthma or COPD. *J Allergy Clin Immunol Pract* 8(5):1606–1613 e1601. <https://doi.org/10.1016/j.jaip.2019.12.019>
- Cates EC, Fattouh R, Johnson JR, Llop-Guevara A, Jordana M (2007) Modeling responses to respiratory house dust mite exposure. *Contrib Microbiol* 14:42–67. <https://doi.org/10.1159/000107054>
- Cevhertas L, Ogulur I, Maurer DJ, Burla D, Ding M, Jansen K, Koch J, Liu C, Ma S, Mitamura Y, Peng Y, Radzikowska U, Rinaldi AO, Satitsuksanoa P, Globinska A, van de Veen W, Sokolowska M, Baerenfaller K, Gao YD, Agache I, Akdis M, Akdis CA (2020) Advances and recent developments in asthma in 2020. *Allergy* 75(12): 3124–3146. <https://doi.org/10.1111/all.14607>
- Chapoval SP, Iijima K, Marietta EV, Smart MK, Chapoval AI, Andrews AG, David CS (2002) Allergic inflammatory response to short ragweed allergenic extract in HLA-DQ transgenic mice lacking CD4 gene. *J Immunol* 168(2):890–899
- Chen W, Sivaprasad U, Tabata Y, Gibson AM, Stier MT, Finkelman FD, Hershey GK (2009) IL-13R alpha 2 membrane and soluble isoforms differ in humans and mice. *J Immunol* 183(12):7870–7876. <https://doi.org/10.4049/jimmunol.0901028>; 183/12/7870 [pii]
- Chiang BL, Chou CC, Ding HJ, Huang MS, Chen JM, Hsieh KH (1995) Establishment of human IgE system in severe combined immunodeficient mice with peripheral blood mononuclear cells from asthmatic children. *J Allergy Clin Immunol* 95(1 Pt 1):69–76; S0091-6749(95)70154-0 [pii]
- Collins RA, Sly PD, Turner DJ, Herbert C, Kumar RK (2003) Site of inflammation influences site of hyperresponsiveness in experimental asthma. *Respir Physiol Neurobiol* 139(1):51–61. S1569904803002283 [pii]
- Conrad ML, Yildirim AO, Sonar SS, Kilic A, Sudowe S, Lunow M, Teich R, Renz H, Garn H (2009) Comparison of adjuvant and adjuvant-free murine experimental asthma models. *Clin Exp Allergy* 39(8): 1246–1254. <https://doi.org/10.1111/j.1365-2222.2009.03260.x>; CEA3260 [pii]
- Corry DB (1999) IL-13 in allergy: home at last. *Curr Opin Immunol* 11(6):610–614; S0952-7915(99)00025-4 [pii]
- Crescitelli R, Lasser C, Lotvall J (2021) Isolation and characterization of extracellular vesicle subpopulations from tissues. *Nat Protoc* 16(3):1548–1580. <https://doi.org/10.1038/s41596-020-00466-1>
- Dearman RJ, Kimber I (2007) A mouse model for food allergy using intraperitoneal sensitization. *Methods* 41(1):91–98. <https://doi.org/10.1016/j.ymeth.2006.07.008>; S1046-2023(06)00139-3 [pii]
- Debeuf N, Haspelslagh E, van Helden M, Hammad H, Lambrecht BN (2016) Mouse models of asthma. *Curr Protoc Mouse Biol* 6(2):169–184. <https://doi.org/10.1002/cpmo.4>

- Dharmage SC, Perret JL, Custovic A (2019) Epidemiology of asthma in children and adults. *Front Pediatr* 7:246. <https://doi.org/10.3389/fped.2019.00246>
- Duez C, Tsicopoulos A, Janin A, Tillie-Leblond I, Thyphronitis G, Marquillies P, Hamid Q, Wallaert B, Tonnel AB, Pestel J (1996) An in vivo model of allergic inflammation: pulmonary human cell infiltrate in allergen-challenged allergic Hu-SCID mice. *Eur J Immunol* 26(5):1088–1093. <https://doi.org/10.1002/eji.1830260520>
- Duez C, Kips J, Pestel J, Tournoy K, Tonnel AB, Pauwels R (2000) House dust mite-induced airway changes in hu-SCID mice. *Am J Respir Crit Care Med* 161(1): 200–206
- Ewart SL, Kuperman D, Schadt E, Tankersley C, Grupe A, Shubitowski DM, Peltz G, Wills-Karp M (2000) Quantitative trait loci controlling allergen-induced airway hyperresponsiveness in inbred mice. *Am J Respir Cell Mol Biol* 23(4):537–545
- Fernandez-Rodriguez S, Ford WR, Broadley KJ, Kidd EJ (2008) Establishing the phenotype in novel acute and chronic murine models of allergic asthma. *Int Immunopharmacol* 8(5):756–763. <https://doi.org/10.1016/j.intimp.2008.01.025>; S1567-5769(08)00045-3 [pii]
- Finkelman FD (2008) Use of unrestrained, single-chamber barometric plethysmography to evaluate sensitivity to cholinergic stimulation in mouse models of allergic airway disease. *J Allergy Clin Immunol* 121(2): 334–335. <https://doi.org/10.1016/j.jaci.2007.11.028>; S0091-6749(07)02266-X [pii]
- Fitzpatrick AM, Higgins M, Holguin F, Brown LA, Teague WG (2010) The molecular phenotype of severe asthma in children. *J Allergy Clin Immunol* 125(4): 851–857 e818. <https://doi.org/10.1016/j.jaci.2010.01.048>; S0091-6749(10)00181-8 [pii]
- Flach TL, Ng G, Hari A, Desrosiers MD, Zhang P, Ward SM, Seamone ME, Vilaysane A, Mucsi AD, Fong Y, Prenner E, Ling CC, Tschopp J, Muruve DA, Amrein MW, Shi Y (2011) Alum interaction with dendritic cell membrane lipids is essential for its adjuvanticity. *Nat Med* 17(4):479–487. <https://doi.org/10.1038/nm.2306>; nm.2306 [pii]
- Fuchs B, Braun A (2008) Improved mouse models of allergy and allergic asthma - chances beyond ovalbumin. *Curr Drug Targets* 9(6):495–502
- Fujita H, Chalubinski M, Rhyner C, Indermitte P, Meyer N, Ferstl R, Treis A, Gomez E, Akkaya A, O'Mahony L, Akdis M, Akdis CA (2011) Claudin-1 expression in airway smooth muscle exacerbates airway remodeling in asthmatic subjects. *J Allergy Clin Immunol* 127(6):1612–1621 e1618. <https://doi.org/10.1016/j.jaci.2011.03.039>; S0091-6749(11)00569-0 [pii]
- Gagnon R, Boutin Y, Hebert J (1995) Lol p I-specific IgE and IgG synthesis by peripheral blood mononuclear cells from atopic subjects in SCID mice. *J Allergy Clin Immunol* 95(6):1268–1275; S0091-6749(95) 70085-4 [pii]
- Glaab T, Hoymann HG, Hohlfeld JM, Korolewicz R, Hecht M, Alarie Y, Tschernig T, Braun A, Krug N, Fabel H (2002) Noninvasive measurement of midexpiratory flow indicates bronchoconstriction in allergic rats. *J Appl Physiol* 93(4):1208–1214. <https://doi.org/10.1152/japplphysiol.01121.2001>
- Glaab T, Mitzner W, Braun A, Ernst H, Korolewicz R, Hohlfeld JM, Krug N, Hoymann HG (2004) Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *J Appl Physiol* 97(3):1104–1111. <https://doi.org/10.1152/japplphysiol.01182.2003>; 01182.2003 [pii]
- Goldberg AL, Rock KL (1992) Proteolysis, proteasomes and antigen presentation. *Nature* 357(6377):375–379. <https://doi.org/10.1038/357375a0>
- Greenough A, Yuksel B, Cheeseman P (2004) Effect of in utero growth retardation on lung function at follow-up of prematurely born infants. *Eur Respir J* 24(5): 731–733. <https://doi.org/10.1183/09031936.04.00060304>; 24/5/731 [pii]
- Hallstrand TS, Lai Y, Ni Z, Oslund RC, Henderson WR Jr, Gelb MH, Wenzel SE (2011) Relationship between levels of secreted phospholipase a(2) groups IIA and X in the airways and asthma severity. *Clin Exp Allergy*. <https://doi.org/10.1111/j.1365-2222.2010.03676.x>
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW (1997) Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 156(3 Pt 1):766–775
- Hamelmann E, Cieslewicz G, Schwarze J, Ishizuka T, Joetham A, Heusser C, Gelfand EW (1999) Anti-interleukin 5 but not anti-IgE prevents airway inflammation and airway hyperresponsiveness. *Am J Respir Crit Care Med* 160(3):934–941
- Hamid Q, Tulic M (2009) Immunobiology of asthma. *Annu Rev Physiol* 71:489–507. <https://doi.org/10.1146/annurev.physiol.010908.163200>
- Hammad H, de Heer HJ, Soullie T, Trottein F, Hoogsteden HC, Lambrecht BN (2004) Activation of peroxisome proliferator-activated receptor-gamma in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma. *Am J Pathol* 164(1):263–271; S0002-9440(10)63116-1 [pii]
- Hauber HP, Gholami D, Meyer A, Pforte A (2003) Increased interleukin-13 expression in patients with sarcoidosis. *Thorax* 58(6):519–524
- Hauk PJ, Krawiec M, Murphy J, Boguniewicz J, Schiltz A, Goleva E, Liu AH, Leung DY (2008) Neutrophilic airway inflammation and association with bacterial lipopolysaccharide in children with asthma and wheezing. *Pediatr Pulmonol* 43(9):916–923. <https://doi.org/10.1002/ppul.20880>
- Hellings PW, Ceuppens JL (2004) Mouse models of global airway allergy: what have we learned and what should we do next? *Allergy* 59(9):914–919. <https://doi.org/10.1111/j.1398-9995.2004.00549.x>; ALL549 [pii]

- Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, Jonas M, Pae C, Wang H, Chi EY (2002) A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 165(1):108–116
- Herz U, Schnoy N, Borelli S, Weigl L, Kasbohrer U, Daser A, Wahn U, Kottgen E, Renz H (1998) A human-SCID mouse model for allergic immune response bacterial superantigen enhances skin inflammation and suppresses IgE production. *J Invest Dermatol* 110(3):224–231. <https://doi.org/10.1046/j.1523-1747.1998.00119.x>; S0022202X97847574 [pii]
- Herz U, Botchkarev VA, Paus R, Renz H (2004a) Increased airway responsiveness, allergy-type-I skin responses and systemic anaphylaxis in a humanized-severe combined immuno-deficiency mouse model. *Clin Exp Allergy* 34(3):478–487; 1887 [pii]
- Herz U, Renz H, Wiedermann U (2004b) Animal models of type I allergy using recombinant allergens. *Methods* 32(3):271–280. <https://doi.org/10.1016/j.ymeth.2003.08.013>; S104620230300241X [pii]
- Hessel EM, Zwart A, Oostveen E, Van Oosterhout AJ, Blyth DI, Nijkamp FP (1995) Repeated measurement of respiratory function and bronchoconstriction in unanesthetized mice. *J Appl Physiol* 79(5):1711–1716
- Hirahara K, Saito S, Serizawa N, Sasaki R, Sakaguchi M, Inouye S, Taniguchi Y, Kaminogawa S, Shiraishi A (1998) Oral administration of a dominant T-cell determinant peptide inhibits allergen-specific TH1 and TH2 cell responses in cry j 2-primed mice. *J Allergy Clin Immunol* 102(6 Pt 1):961–967. S0091674998004412 [pii]
- Hirose K, Iwata A, Tamachi T, Nakajima H (2017) Allergic airway inflammation: key players beyond the Th2 cell pathway. *Immunol Rev* 278(1):145–161. <https://doi.org/10.1111/imr.12540>
- Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, Herszberg B, Lavoie JP, McVicker CG, Moir LM, Nguyen TT, Peng Q, Ramos-Barbon D, Stewart AG (2004) Proliferative aspects of airway smooth muscle. *J Allergy Clin Immunol* 114(2 Suppl): S2–S17. <https://doi.org/10.1016/j.jaci.2004.04.039>; S0091674904014125 [pii]
- Holgate ST (2008) Pathogenesis of asthma. *Clin Exp Allergy* 38(6):872–897. <https://doi.org/10.1111/j.1365-2222.2008.02971.x>; CEA2971 [pii]
- Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL (2000) Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 105(2 Pt 1): 193–204. S0091674900606255 [pii]
- Irvine CGCR (1987) Pathophysiology and physiological assessment of the asthmatic patient. *Semin Respir Med* 8:201–215
- Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, Ueyama Y, Koyanagi Y, Sugamura K, Tsuji K, Heike T, Nakahata T (2002) NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100(9): 3175–3182. <https://doi.org/10.1182/blood-2001-12-0207>
- Jarnicki AG, Tsuji T, Thomas WR (2001) Inhibition of mucosal and systemic T(h)2-type immune responses by intranasal peptides containing a dominant T cell epitope of the allergen Der p 1. *Int Immunol* 13(10): 1223–1231
- Jensen-Jarolim E, Roth-Walter F, Jordakieva G, Pali-Scholl I (2021) Allergens and adjuvants in allergen immunotherapy for immune activation, tolerance, and resilience. *J Allergy Clin Immunol Pract* 9(5): 1780–1789. <https://doi.org/10.1016/j.jaip.2020.12.008>
- Jin H, He R, Oyoshi M, Geha RS (2009) Animal models of atopic dermatitis. *J Invest Dermatol* 129(1):31–40. <https://doi.org/10.1038/jid.2008.106>; jid2008106 [pii]
- Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, Gutierrez-Ramos JC, Ellis R, Inman MD, Jordana M (2004) Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 169(3):378–385. <https://doi.org/10.1164/rccm.200308-1094OC>; 200308-1094OC [pii]
- Jutel M, Akdis CA (2011) T-cell subset regulation in atopy. *Curr Allergy Asthma Rep* 11(2):139–145. <https://doi.org/10.1007/s11882-011-0178-7>
- Karol MH (1994) Animal models of occupational asthma. *Eur Respir J* 7(3):555–568
- Kenyon NJ, Kelly EA, Jarjour NN (2000) Enhanced cytokine generation by peripheral blood mononuclear cells in allergic and asthma subjects. *Ann Allergy Asthma Immunol* 85(2):115–120. [https://doi.org/10.1016/S1081-1206\(10\)62450-7](https://doi.org/10.1016/S1081-1206(10)62450-7); S1081-1206(10)62450-7 [pii]
- Kool M, Willart MA, van Nimwegen M, Bergen I, Pouliot P, Virchow JC, Rogers N, Osorio F, Reis ESC, Hammad H, Lambrecht BN (2011) An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. *Immunity* 34(4):527–540. <https://doi.org/10.1016/j.immuni.2011.03.015>; S1074-7613(11)00122-1 [pii]
- Kumar RK, Foster PS (2002) Modeling allergic asthma in mice: pitfalls and opportunities. *Am J Respir Cell Mol Biol* 27(3):267–272
- Kumar RK, Herbert C, Foster PS (2008) The “classical” ovalbumin challenge model of asthma in mice. *Curr Drug Targets* 9(6):485–494
- Kurup VP, Choi H, Murali PS, Resnick A, Fink JN, Coffman RL (1997) Role of particulate antigens of *Aspergillus* in murine eosinophilia. *Int Arch Allergy Immunol* 112(3):270–278
- Leigh R, Ellis R, Wattie J, Southam DS, De Hoogh M, Gauldie J, O’Byrne PM, Inman MD (2002) Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am J Respir Cell Mol Biol* 27(5):526–535
- Lex C, Payne DN, Zacharasiewicz A, Li AM, Wilson NM, Hansel TT, Bush A (2005) Sputum induction in children with difficult asthma: safety, feasibility, and

- inflammatory cell pattern. *Pediatr Pulmonol* 39(4): 318–324. <https://doi.org/10.1002/ppul.20159>
- Lilly CM, Tateno H, Oguma T, Israel E, Sonna LA (2005) Effects of allergen challenge on airway epithelial cell gene expression. *Am J Respir Crit Care Med* 171(6): 579–586. <https://doi.org/10.1164/rccm.200404-532OC>; 200404-532OC [pii]
- Lloyd CM (2007) Building better mouse models of asthma. *Curr Allergy Asthma Rep* 7(3):231–236
- Lodrup Carlsen KC, Carlsen KH (2001) Effects of maternal and early tobacco exposure on the development of asthma and airway hyperreactivity. *Curr Opin Allergy Clin Immunol* 1(2):139–143
- Matsuda A, Hirakata T, Asada Y, Nakae S (2021) Experimental mouse models of ragweed- and papain-induced allergic conjunctivitis. *Methods Mol Biol* 2223:133–149. https://doi.org/10.1007/978-1-0716-1001-5_10
- Maunsell K, Wraith DG, Cunnington AM (1968) Mites and house-dust allergy in bronchial asthma. *Lancet* 1(7555):1267–1270
- McAllen MK, Assem ES, Maunsell K (1970) House-dust mite asthma. Results of challenge tests on five criteria with *Dermatophagoides pteronyssinus*. *Br Med J* 2(5708):501–504
- McCune JM (1991) SCID mice as immune system models. *Curr Opin Immunol* 3(2):224–228
- McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH (2001) Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nat Immunol* 2(12):1109–1116. <https://doi.org/10.1038/ni739>; ni739 [pii]
- McMillan SJ, Lloyd CM (2004) Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 34(3):497–507; 1895 [pii]
- McMillan SJ, Bishop B, Townsend MJ, McKenzie AN, Lloyd CM (2002) The absence of interleukin 9 does not affect the development of allergen-induced pulmonary inflammation nor airway hyperreactivity. *J Exp Med* 195(1):51–57
- Melgar S, Karlsson A, Michaelsson E (2005) Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. *Am J Physiol Gastrointest Liver Physiol* 288(6):G1328–G1338. <https://doi.org/10.1152/ajpgi.00467.2004>; 00467.2004 [pii]
- Meyer N, Zimmermann M, Burgler S, Bassin C, Woehrl S, Moritz K, Rhyner C, Indermitte P, Schmid-Grendelmeier P, Akdis M, Menz G, Akdis CA (2010) IL-32 is expressed by human primary keratinocytes and modulates keratinocyte apoptosis in atopic dermatitis. *J Allergy Clin Immunol* 125(4):858–865 e810. <https://doi.org/10.1016/j.jaci.2010.01.016>; S0091-6749(10)00041-2 [pii]
- Moeller A, Carlsen KH, Sly PD, Baraldi E, Piacentini G, Pavord I, Lex C, Saglani S, Children ERSTFMAi (2015) Monitoring asthma in childhood: lung function, bronchial responsiveness and inflammation. *Eur Respir Rev* 24(136):204–215. <https://doi.org/10.1183/16000617.00003914>
- Mojtabavi N, Dekan G, Stingl G, Epstein MM (2002) Long-lived Th2 memory in experimental allergic asthma. *J Immunol* 169(9):4788–4796
- Mosier DE, Gulizia RJ, Baird SM, Wilson DB (1988) Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* 335(6187):256–259. <https://doi.org/10.1038/335256a0>
- Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17(3):138–146; 0167569996806062 [pii]
- Nelde A, Teufel M, Hahn C, Duschl A, Sebald W, Brocker EB, Grunewald SM (2001) The impact of the route and frequency of antigen exposure on the IgE response in allergy. *Int Arch Allergy Immunol* 124(4):461–469. 53781 [pii]
- Nials AT, Uddin S (2008) Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech* 1(4-5):213–220. <https://doi.org/10.1242/dmm.000323>
- Niederhorn JY (2008) Immune regulatory mechanisms in allergic conjunctivitis: insights from mouse models. *Curr Opin Allergy Clin Immunol* 8(5):472–476. <https://doi.org/10.1097/ACI.0b013e32830edbc9>; 00130832-200810000-00020 [pii]
- Palecek F, Palecekova M, Aviado DM (1967) Emphysema in immature rats. Condition produced by tracheal constriction and papain. *Arch Environ Health* 15(3): 332–342
- Periz M, Perez-Cano FJ, Rodriguez-Lagunas MJ, Cambras T, Pastor-Soplin S, Best I, Castell M, Massot-Cladera M (2020) Development and characterization of an allergic asthma rat model for interventional studies. *Int J Mol Sci* 21(11). <https://doi.org/10.3390/ijms21113841>
- Perros F, Hoogsteden HC, Coyle AJ, Lambrecht BN, Hammad H (2009) Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation. *Allergy* 64(7): 995–1002. <https://doi.org/10.1111/j.1398-9995.2009.02095.x>; ALL2095 [pii]
- Persson CG, Erjefalt JS, Korsgren M, Sundler F (1997) The mouse trap. *Trends Pharmacol Sci* 18(12): 465–467; S0165-6147(97)01142-5 [pii]
- Pestel J, Jeannin P, Delneste Y, Dessaint JP, Cesbron JY, Capron A, Tscicopoulos A, Tonnel AB (1994) Human IgE in SCID mice reconstituted with peripheral blood mononuclear cells from *Dermatophagoides pteronyssinus*-sensitive patients. *J Immunol* 153(8): 3804–3810
- Petak F, Habre W, Donati YR, Hantos Z, Barazzone-Argiroffo C (2001) Hyperoxia-induced changes in mouse lung mechanics: forced oscillations vs. barometric plethysmography. *J Appl Physiol* 90(6):2221–2230
- Pivniouk V, Gimenes Junior JA, Honeker LK, Vercelli D (2020) The role of innate immunity in asthma

- development and protection: lessons from the environment. *Clin Exp Allergy* 50(3):282–290. <https://doi.org/10.1111/cea.13508>
- Rankin JA, Picarella DE, Geba GP, Temann UA, Prasad B, DiCosmo B, Tarallo A, Stripp B, Whitsett J, Flavell RA (1996) Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci U S A* 93(15):7821–7825
- Ray A, Oriss TB, Wenzel SE (2015) Emerging molecular phenotypes of asthma. *Am J Physiol Lung Cell Mol Physiol* 308(2):L130–L140. <https://doi.org/10.1152/ajplung.00070.2014>
- Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW (1992) Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J Allergy Clin Immunol* 89(6):1127–1138; 0091-6749 (92)90296-E [pii]
- Romagnani S (1994) Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 12:227–257. <https://doi.org/10.1146/annurev.iy.12.040194.001303>
- Ruckert R, Brandt K, Braun A, Hoymann HG, Herz U, Budagian V, Durkop H, Renz H, Bulfone-Paus S (2005) Blocking IL-15 prevents the induction of allergen-specific T cells and allergic inflammation in vivo. *J Immunol* 174(9):5507–5515; 174/9/5507 [pii]
- Sagar S, Akbarshahi H, Uller L (2015) Translational value of animal models of asthma: challenges and promises. *Eur J Pharmacol* 759:272–277. <https://doi.org/10.1016/j.ejphar.2015.03.037>
- Sarpong SB, Zhang LY, Kleeberger SR (2003) A novel mouse model of experimental asthma. *Int Arch Allergy Immunol* 132(4):346–354. <https://doi.org/10.1159/000074902>; IAA2003132004346 [pii]
- Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobraaten L, Rajan TV, Greiner DL et al (1995) Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J Immunol* 154(1):180–191
- Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R (2005) Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* 174(10):6477–6489; 174/10/6477 [pii]
- Shultz LD, Ishikawa F, Greiner DL (2007) Humanized mice in translational biomedical research. *Nat Rev Immunol* 7(2):118–130. <https://doi.org/10.1038/nri2017>; nri2017 [pii]
- Sonar SS, Hsu YM, Conrad ML, Majeau GR, Kilic A, Garber E, Gao Y, Nwankwo C, Willer G, Dudda JC, Kim H, Bailly V, Pagenstecher A, Rennert PD, Renz H (2010) Antagonism of TIM-1 blocks the development of disease in a humanized mouse model of allergic asthma. *J Clin Invest* 120(8):2767–2781. <https://doi.org/10.1172/JCI39543>; 39543 [pii]
- Song HK, Hwang DY (2017) Use of C57BL/6N mice on the variety of immunological researches. *Lab Anim Res* 33(2):119–123. <https://doi.org/10.5625/lar.2017.33.2.119>
- Steinke JW, Lawrence MG, Teague WG, Braciale TJ, Patrie JT, Borish L (2021) Bronchoalveolar lavage cytokine patterns in children with severe neutrophilic and paucigranulocytic asthma. *J Allergy Clin Immunol* 147(2):686–693 e683. <https://doi.org/10.1016/j.jaci.2020.05.039>
- Steinsvik TE, Aaberge IS, Gaarder PI, Lovik M (1999) Interleukin-13 and human immunoglobulin E production in severe combined immunodeficiency mice transplanted with human peripheral blood lymphocytes. *Scand J Immunol* 49(1):67–72
- Stevenson CS, Birrell MA (2010) Moving towards a new generation of animal models for asthma and COPD with improved clinical relevance. *Pharmacol Ther*. <https://doi.org/10.1016/j.pharmthera.2010.10.008>; S0163-7258(10)00215-9 [pii]
- Takeda K, Gelfand EW (2009) Mouse models of allergic diseases. *Curr Opin Immunol* 21(6):660–665. <https://doi.org/10.1016/j.coi.2009.09.005>; S0952-7915(09)00181-2 [pii]
- Taussig LM, Wright AL, Morgan WJ, Harrison HR, Ray CG (1989) The Tucson Children's respiratory study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. *Am J Epidemiol* 129(6):1219–1231
- Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK (1998) An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 53(10):849–856
- Tomkinson A, Duez C, Cieslewicz G, Pratt JC, Joetham A, Shanafelt MC, Gundel R, Gelfand EW (2001) A murine IL-4 receptor antagonist that inhibits IL-4 and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness. *J Immunol* 166(9):5792–5800
- Tournoy KG, Kips JC, Schou C, Pauwels RA (2000) Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy* 30(1):79–85; cea772 [pii]
- Ulrich K, Hincks JS, Walsh R, Wetterstrand EM, Fidock MD, Sreckovic S, Lamb DJ, Douglas GJ, Yeadon M, Perros-Huguet C, Evans SM (2008) Anti-inflammatory modulation of chronic airway inflammation in the murine house dust mite model. *Pulm Pharmacol Ther* 21(4):637–647. <https://doi.org/10.1016/j.pupt.2008.02.006>; S1094-5539(08)00033-3 [pii]
- Van Hove CL, Maes T, Cataldo DD, Gueders MM, Palmans E, Joos GF, Tournoy KG (2009) Comparison of acute inflammatory and chronic structural asthma-like responses between C57BL/6 and BALB/c mice.

- Int Arch Allergy Immunol 149(3):195–207. <https://doi.org/10.1159/000199715>; 000199715 [pii]
- Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ (2002) The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol* 26(2):202–208
- Vijayaraghavan R, Schaper M, Thompson R, Stock MF, Alarie Y (1993) Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch Toxicol* 67(7):478–490
- Wagner JG, Harkema JR (2007) Rodent models of allergic rhinitis: relevance to human pathophysiology. *Curr Allergy Asthma Rep* 7(2):134–140
- Wegmann M (2008) Animal models of chronic experimental asthma – strategies for the identification of new therapeutic targets. *J Occup Med Toxicol* 3(Suppl 1):S4. <https://doi.org/10.1186/1745-6673-3-S1-S4>; 1745-6673-3-S1-S4 [pii]
- Wenzel S, Holgate ST (2006) The mouse trap: it still yields few answers in asthma. *Am J Respir Crit Care Med* 174(11):1173–1176; discussion 1176–1178. <https://doi.org/10.1164/rccm.2609002>; 174/11/1173 [pii]
- Wiedermann U, Jahn-Schmid B, Lindblad M, Rask C, Holmgren J, Kraft D, Ebner C (1999a) Suppressive versus stimulatory effects of allergen/cholera toxoid (CTB) conjugates depending on the nature of the allergen in a murine model of type I allergy. *Int Immunol* 11(10):1717–1724
- Wiedermann U, Jahn-Schmid B, Repa A, Kraft D, Ebner C (1999b) Modulation of an allergic immune response via the mucosal route in a murine model of inhalative type-I allergy. *Int Arch Allergy Immunol* 118(2-4): 129–132; 24048 [pii]
- Williams CM, Galli SJ (2000) Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 192(3):455–462
- Winkler B, Baier K, Wagner S, Repa A, Eichler HG, Scheiner O, Kraft D, Wiedermann U (2002) Mucosal tolerance as therapy of type I allergy: intranasal application of recombinant bet v 1, the major birch pollen allergen, leads to the suppression of allergic immune responses and airway inflammation in sensitized mice. *Clin Exp Allergy* 32(1):30–36; 1214 [pii]
- Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA (1999) Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 103(6):779–788. <https://doi.org/10.1172/JCI5909>
- Zosky GR, Sly PD (2007) Animal models of asthma. *Clin Exp Allergy* 37(7):973–988. <https://doi.org/10.1111/j.1365-2222.2007.02740.x>; CEA2740 [pii]