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Animal Model of Asthma, Various Methods and Measured Parameters: A Methodological Review

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ABSTRACT

Asthma is a chronic inflammatory disease of the airway with extensive airway remodeling. The ethical issues associated with the studies in asthmatic patients, required development of animal model of asthma. Animal models of asthma can provide valuable information on several features of asthma pathogenesis and treatment. Although these models cannot carry out all clinical features, they are valuable to understand mechanisms of the disease and curative access.

Related articles were searched in different databases from September 1994 to April 2016 using; animal model of asthma, animal sensitization, allergen-induced asthma in animals terms.

Although there are several reviews on this topic, in the present article, induction of animal model of asthma in different animals, various methods used for this purpose, measured parameters and research purposes were reviewed, which will help investigators to use the appropriate animal, methods, and evaluating parameters depending on their study design.

In this study various method used for induction of animal model of asthma in different animals and measured parameters were described, which will help investigators to use the appropriate animal, method and evaluating parameters depending on their study design.

Keywords: Asthma; Animal models; Airway remodeling; Airway inflammation; Airway responsiveness

INTRODUCTION

Asthma is characterized by reversible airway obstruction, increased airway responsiveness, enhanced mucus production, airway inflammation and remodeling of the airways.[1] Remodeling of the airways in asthma includes: epithelial fibrosis, goblet cell metaplasia and hyperplasia, mucus hyper-secretion as well as hypertrophy and hyperplasia of airway smooth muscle, which depends on the repeated exposure to the allergen, and airway inflammation severity and

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duration.[2]

Asthma is a disease with different phenotypes but there is no standard way to define its phenotypes.[3] Different phenotypes of asthma include not only clinical parameters, but also should include other biomarkers to find genetic and endotypic differences.[4] Cluster analyses of asthmatic patients have discovered various specific phenotypes, which were repeated in animal models.[5] Phenotypic categories of asthma include phenotypes defined by clinical or physiological criteria; phenotypes related to environmental triggers; and phenotypes defined by their pathobiology. Different clinical phenotypes of asthma are defined based on the severity of asthma (moderate to severe), response to treatment, the frequency of exacerbations, the presence of airflow limitation, and age onset of asthma. These patients are resistant to corticosteroids due to a defect in response to the drug. In defined phenotype by the frequency of exacerbations, some patients are prone to repeated exacerbations. These patients may have relatively normal lung function, low lung function or severe changes in lung function. In case of defined phenotype by airflow limitation, patients with marked airflow limitation have only moderately symptomatic or exacerbation-prone disease. Finally, it seems that the age onset of asthma provides a variety of disease's phenotypes. Patients with early-onset of asthma (asthma onset before 12 years of age) are more likely to develop allergic sensitivities compared to patients with late-onset asthma.[3]

Trigger-related phenotypes include allergic asthma, occupational asthma, menses-related asthma and exercise-induced asthma. Allergic sensitization which triggers asthma might be the largest overall phenotype in childhood asthma and asthmatic adults.[3,6] In addition, three pathological phenotypes of asthma also have been suggested on the basis of the predominant cell type involved: eosinophilic, neutrophilic, and paucigranulocytic.[7]

A limitation of animal models is that they cannot mimic all features and various phenotypes of the disease[8] but can represent many inflammatory, structural and physiological features.[5] For example, most animal models of asthma are based on a Th2-drived phenotype, while half of asthma patients suffer from airway disorders without Th2-mediated immune response.[8,9] Therefore to create models that are able to reflect the specific phenotype of asthmatic patients, new clinical information is required.[5] Proper use of these models leads to identifying mechanisms, cells and new pathways which result in a phenotype consistent with human phenotypes.[10]

The change in airway wall structure influences the airflow which may be linked to and airway hyper-responsiveness, the main characteristic feature of asthma.[2] Despite the intensive efforts, the pathobiology of asthma is still poorly understood.[11] Conducting the required studies in asthmatic patients to understand the underlying mechanisms of asthma, to identify important pathways and drug therapies is not possible due to ethical reasons. Therefore, the development of animal models of asthma is required to perform studies on underling mechanisms and development of the asthma disease.[12]

There are a wide range of asthma models in different animals and using various methods for research purposes. Animal models of asthma represent applicable tools for understanding disease pathophysiology and testing potential drug therapies. The results of these models could be applied on asthmatics patients depending on the species of the animal chosen and the method of induction of the disease, [13](Figure 1).

In this review, induction of animal model of asthma in different animals, various method used for this purpose, and measured parameters were reviewed, which will help investigators to use the appropriate animal, method and evaluating parameters depending on their study design (Figure 2).

MATERIALS AND METHOD

Related articles were searched in different databases including; Google scholar, PubMed, and Science direct from September 1994 to April 2016. Key search terms were animal model of asthma, animal sensitization, and allergen-induced asthma in animals, as well as pathophysiological changes in asthmatic animals.[14]

Different Methods, Using Various Animals for Induction of Animal Models of Asthma

The development of animal models of asthma involves a process of animal sensitizing to an antigen followed by effects on the airways in order to present allergic responses. The physiological and immunological airway responses could be different between species based on the method of sensitization and antigen used.[15] Extracts or protein derived from

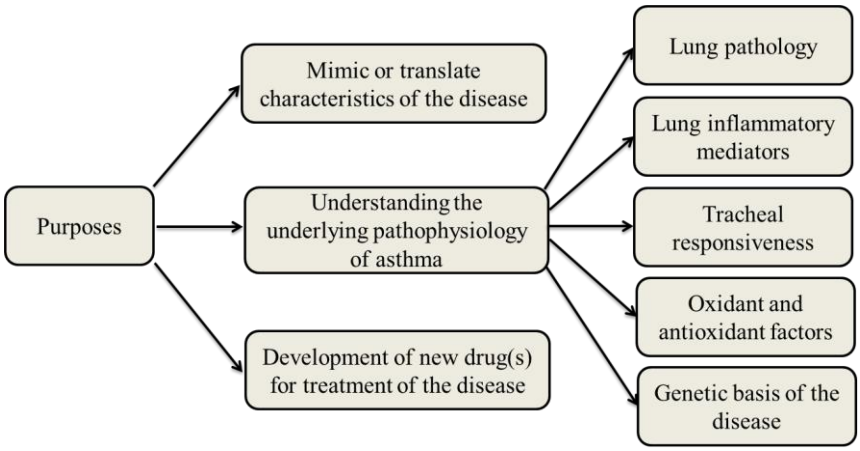

Figure 1. Various purposes of inducing animal model of asthma

potent allergens including cockroach, ragweed, or fungi have been increasingly used as inducers of animal model of asthma in mice and other species. In this section different inducers of experimental models of asthma in various animals is reviewed (Table 1).

Ovalbumin (OVA)

An adjuvant, usually alum (potassium aluminum sulfate), may also be used. Sensitized animals are then challenged with a secondary exposure by either dermal, inhalation, or airway instillation. In addition, the volume or concentrations as well as the time of exposure to allergen (several days or weeks) could be different.[16] There are many different sensitization protocols such as acute or chronic asthma models which could be induced in animals.[17] Acute sensitization protocols usually require multiple systemic administration of allergen in the presence of an adjuvant. Aluminum hydroxide (AlOH3) is one of the best choices for the development of the Th2 immune response when the animals are exposed to antigen.

OVA has been used as an allergen in some experimental animal models of asthma.[18] It is an allergen derived from chicken egg, which induces allergic pulmonary inflammation in laboratory rodents.[19] OVA models have proved limited success and only modest pulmonary inflammation and airway hyper-responsiveness (AHR) have been observed.[20]

Mice

Mouse is the most common species studied in animal models of asthma. Principally, mice are sensitized to allergen with alum as an adjuvant via intraperitoneal (IP) injection.[21] There are numerous mouse-specific probes for studying allergic results and they are proportionately cheap.[11] Mice are easily sensitized by many antigens including OVA, to which they are not normally exposed. One of the major drawbacks of the mouse models is the lack of chronic response to allergen following sensitization.[22] OVA challenge models of asthma offer many opportunities for increasing our understanding of the pathogenetic mechanisms of this disease.[18] In some studies male/female C57BL/6 or BALB/c mice were

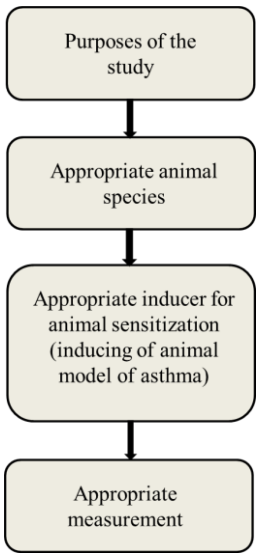

Figure 2. Different steps needed for inducing animal model of asthma.

Table 1. Different methods used for induction of animal model of asthma

Inducer	Animals	Methods	Ref.
OVA	**Mice**	- IP 100-µL, 50 µL OVA (200 µL/mL)+50 µL Al(OH)3, days 0 and 14, expose to 1% OVA 30 min/day, 3 days	(23)
		- IP 100 µg OVA+20 mg Al(OH)3 on day 1, IT 250 µg OVA on day 9, 125 µg OVA on days 16, 19, 22.	(24)
		- IP 20 µg OVA+2 mg Al(OH)3 on days 0 and 7, expose to 1% OVA aerosol on days 21 and 25, 30 min/day	(25)
		- IP 1 mL of 10% OVA on day 1, expose to 1% OVA aerosol for 14 successive days, 30 min/day.	(26)
		- IP 20 mg OVA+2 mg Al(OH)3, days 1 and 14, expose to 100 mg OVA/mL aerosol, days 28-30, 20 min/day	(27)
		- IP 10 µg OVA+Al(OH)3, days 1 and 8, expose to 100 µg OVA, days 22, 29, 36, 43, 50, 52, 20 min	(28)
		- IP 50 µg OVA+4 mg Al(OH)3 on days 0, 7, 14, OVA expose to OVA3% in PBS, days 21-27, 30 min/day	(29)
		- IP 100 µg OVA on days 1 and 14, in OVA 100 µg on days 14, 25, 26 and 27	(30)
		- IP 100 µg OVA+Al(OH)3 on the first day, i.n OVA challenges on days 8, 15, 18 and 21	(31)
		-IP 10 µg OVA+Al(OH)3, IG 20 mg OVA in water on day 27 to day 29	(32)
		- SC 25 mg OVA+1mg Al(OH)3,days 0,7,14, 21, i.n OVA (20 ng/50 mL PBS), days 27, 29, 2/week, 3 months	(33, 34)
	Rat	- IP 1 mg OVA+20 mg $Al(OH)_3$ on days 0, 7 and 14, IT 1.1% OVA on day 21	(37)
		- IP 20 µg/mL OVA in PBS+ Al(OH)3 on days 0 and 14, expose to OVA 1% for 1 h on days 22, 23 and 24.	(40)
		- SC 1 mg OVA on days 1, 3, 7, 14 after birth, expose to 20 g/L aerosol for 7 continues days, 20 min/day	(42)
		- IP 1 mg OVA+200 µg Al(OH)3 on days 0 and 7, expose to OVA 1% every two days, days 14-70, 30 min.-	(38)
		-IP 1 mg OVA+100 µg Al(OH)3 days 1, 2, 3, expose to OVA 1% aerosol, days 6, 9, 12, 15, 18, 21, 20 min	(39)
		- IP 1 mg OVA+50 mg $Al(OH)_3$ in 0.5 mL salin, after 1 week, expose to 0.02 mg OVA+50 mg $Al(OH)_3$ in 0.5 mL salin IP from day 14, expose to aesrosol of 4% OVA for 18±1 days, 5 min daily	(41)
	Guinea pig	- Expos to OVA aerosol (1%) for14 days, IP OVA 10% (1 mL) on day 15	(60)
		- IP 100 mg and SC100 mg OVA on days 0 and 14	(51)
		- IP 1 mg OVA on day 1, expos to 63 mg/L OVA on Day 8, 15 and 29, for 6 min.	(50)
		- IP and SC100 mg (1mL) OVA on day 1, IP 10 mg OVA on day 8, expose to aerosol 4% OVA from day 14, for 18 days, 4 mins/day	(47,53,54,92,113,139)
		- IP 10 mg OVA+100 mg Al(OH3) on day 1 and 2 mg OVA+100 mg Al (OH)3 on day 7, expose to 4% OVA aerosol from day 14 for 18±1 days, 5 min/day	(52, 55, 56)
		- IP 10 µg OVA+100 mg Al(OH)3 (in 1 mL saline) on day 14 or 21, expos to 1% OVA aerosol for 2 min (macroshock) or 0.01% OVA for 60 min (microshock) on days 14-21	(57)
		- IP 20 mg·kg -1 OVA on days 1, 3 and 6, expose to aerosol of 5% OVA for 10 min	(58)
		- IP 20 µg OVA+10 mg Al(OH)3 on days 0, 2 and 4, expos to OVA aerosol (5 mg/mL 0.9% saline)	(59)
	Rabit	- IP 10 mg OVA twice on days 1 and 14 →chronic asthma	(63)
		- IP 0.1 mg OVA+10 mg Al(OH)3, days 1 & 14, expose to OVA (10 mg/mL) aerosol, days 28-30, 10 min/day	(64)

		Sheep	- SC OVA (100 mg/1 mL saline+50 µl Al (OH)3) 3 times at 2-week intervals, 'boost' SC after 4 weeks	(69)
		Dog	- SC 1 µg OVA (100 µL, OVA of 10 µg/mL saline) monthly for 6 months, booster SC 1 µg OVA 2–7 times/year (intervals between injections 2 weeks - 16 months).	(66)
			- SC OVA (100 mg), expose to OVA aerosol (5%) for 1 week, 15 min/day	(67)
		Horse	- IM 10 mg OVA on days 0 and 10, IT (5 mg OVA/1 mL saline) on day 20.	(71)
			- Expose to OVA 4% aerosol (15 min/daily) for 30 days	(72)
HDM		**Mice**	- IN 25 µg/10 µl saline, 5 consecutive days/week, 7 weeks	(75)
			- IN 25 µg/10 µl saline, 5 consecutive days/week, 5 weeks	(76)
		Sheep	- Intramammary 1 mg solubilized HDM every 2 weeks for 3-4 weeks	(154)
			- SC HDM (50 µg/1 mL saline+Al(OH)3,2week	(69)
		Monkey	- IP (3.6 mL/kg)+IM (0.4 mL/kg) 3 times weekly, IM 0.4 mL/kg +5mg/50mg/mL Al(OH)3, 5weeks later	(78)
Other agent	**C. Ext.**	**Mice**	- IP 2.5 µg CRA or 2.5 µg HDM + Al(OH)3) on day 0, 14 and 21, IT 20 µg CRA or HDM twice a week for seven consecutive weeks from day 26	(80)
	Blag2, Derf1	**Mice**	-IP Blag2 (0.05 µg)+Derf1 (0.05 µg)+0.2 mL Al(OH)3, days 0, 6, 14, O.T Blag2 (25 µg) and Der f 1 (25 µg)	(83)
	Latex Ext	**Mice**	-IP 100 µg latex extract 1/week for two weeks, expose to 50 µg latex in 30 µl PBS 2/week for 4 weeks	(81)
	DRA	**Mice**	-SC DRA allergens (5 µg dust mite, 50 µg ragweed, and 5 µg A. species) +100 µL Al(OH)3) in normal saline on day 1 and day 8. i.n. DRA allergens twice a week for 8 weeks from week 3 to week 10	(82)
	Ascaris sum	**Rat**	- inhalation DNP-As, 2 mg protein for 5 min, boosted by DNP-As (0.5 mg protein) 5 days later	(35)
		Cat	- IM adjuvant–allergen emulsion 2 times, 2 weeks apart, a single 5-min expos to 0.01% A. sum aerosol	(155)
		Pig	- Expose to A. sum extract (7 $mg \cdot mL^{-1}$ /2 mL saline), via tracheal tube to the lower airways, 5 min/day	(156)
		Dog	- Expose to A. sum aerosol serially diluted in saline (1, 10, 10^2, lo^3, and lo^4 PNUiml, airflow of 5 L/min. A), 5 min via an endotracheal tube, and 5 min rest between each challenge for 2 weeks	(157)
	C. Dust	**Gui. pig**	- ID. 0.1 mL 0.3% C. Dust /corn oil, expos to C. Dust aerosol (0.5 mg/mL), 21-28 days later, 10 min/2 hours,	(158)
	Ragweed	**Dog**	- Instilled Ragweed [0.30 mg/3 mL salin] in right cardiac sub segment+diaphragmatic lobes by broncscop	(159)
	LPS	**Rat**	- Expose to 1 mg/mL LPS/PBS (pH 7.4), 15 min, turning off the nebulizer, plugged the chamber inlet and outlets, rats remained in the chamber for further 15 min to breathe the remaining aerosolized solution.	(160)

OVA: Ovalbumin; HDM: House Dust Mite; C. Ext.: Cockroach extracts; A. Antig.: Ascaris antigen; C. Dust: Cotton dust; IP: intraperitoneal; IT: intra tracheal; IN: intranasal; OT: oro tracheal; SC: subcutaneous; Expos: Exposure; Ref.: References. Phosphate buffered saline (PBS); DNP-As: 2,4-dinitrophenylated Ascaris extract; IM: intramuscular; ID.: intradermally; DRA: Allergens including extracts of house dust mite, ragweed and Aspergillums species. IG: intra-gastric; Blag2: recombinant Blatella germanica 2 (cockroach allergen); Derf1: Dermatophagoides farinae 1 (house dust mite allergen).

systemically sensitized to OVA and chronically challenged with low particle mass concentrations of aerosolized OVA. The main methods for sensitization of mice to OVA included IP. injection of OVA followed by exposure to OVA aerosol.[23-30] The other methods for sensitization of mice to OVA are IP injection of OVA followed by intranasal OVA challenge[31] or IP injection of OVA followed by intra-gastric OVA challenge.[32] In addition, subcutanous (SC) injection of OVA followed by intranasal OVA

challenge was used in other studies.[33,34] Different methods for sensitization of mice to OVA are summarized in Table 1.

Rat

Rats are also common as models of allergic airways disease. They are relatively cheap similar to mice, which allows extensive studies to be conducted.[22] The larger size and higher stability of rats under anesthesia is an advantage in measuring physiological results such as acute responses to allergen inhalation.[35] A disadvantage of the rat and mouse models of asthma, is the difficulty in performing protocols, which result in the chronic changes in the airways associated with asthma.[36] However, they have been useful in understanding the mechanisms of asthma and modulation of tolerance in allergy.[36] In one of the experimental studies, male Wistar rats were sensitized IP with 1 mg OVA added on 20 mg $Al(OH)_3$ gelatinous on days 0, 7, and 14. They were challenged with 1.1% OVA in 200 µL normal saline by intra-tracheal (IT) instillation on day 21.[37] Female/male Wistar rats could be sensitized by IP. injection and then exposed to OVA aerosol.[38-41] In addition, IP injection followed by IT OVA challenge[37] and SC. injection of OVA followed by exposure to OVA aerosol[42] were also used for this purpose. Table 1 summarizes different methods of rat sensitization to OVA.

Guinea Pig

Guinea pigs are among the oldest animal models of allergic airway responses and studies on these animals have been done for one century.[43] Compared to rodent models of asthma, guinea pigs are readily sensitized to OVA and it is easy to bring out a response that is similar to an asthmatic phenotype and increased airway responsiveness.[44] The guinea pig is perfect to be used as a model for studying hypersensitivity to chemical irritant factors.[14] Guinea pigs are also often used as a screening model for drug therapy in asthma and have been useful in the development of drugs such as beta receptor agonists and corticosteroids.[45] The response of isolated guinea pig airways to pharmacological agonists has been compared with humans and they are a good model for human airways responsiveness.[44] It was found that there were similar responses in guinea pig and human airways when exposed to methacholine (Mch) and histamine.[46] Studies with guinea pig showed increased IgG and IgE in response to allergen and hyper- reactivity reaction resulted from allergen sensitization.[47] Another utility of the asthma model in guinea pigs is the eosinophilic and neutrophilic pulmonary infiltration.[48] In order to induce an asthma model in these animals, guinea pigs are often pretreated with anti-histamines.[49] In some studies guinea pigs were sensitized by IP injection of OVA and challenged with OVA aerosol 3 weeks later. All animals developed severe immediate-onset airway constrictive responses.[50] In most studies guinea pigs were sensitized by IP. injection followed by exposure to OVA aerosol.[47,50-59] In other method for this purpose, male Hartley guinea pigs were first exposed to OVA aerosol followed by its IP injection.[60] In Table 1, different methods for sensitization of guinea pigs to OVA are summarized.

Other Animals

The rabbit resembles humans when lung is the target organ for anaphylactic response. This species can demonstrate both early and late reactions.[61.62] The late reactions associated with inflammation are thought to be important in development of asthma.[61] The rabbit is a valuable model since it also produces IgE as the primary anaphylactic antibody. Rabbits were also sensitized to OVA by IP injection followed by exposure to OVA aerosol in few studies.[63,64]

Dogs have been also used as an animal model of asthma. It has been suggested that dogs represent an ideal model of allergy as they have a natural trait to develop allergic responses to antigens that are clinically significant to humans.[65] This allergy usually manifests itself in superficial reactions in the form of dermatitis or conjunctivitis and reactions in the airways like asthma.[65] Two models for sensitization of dogs with OVA were used including: 1) SC. injection of OVA for several months[66] and 2) SC injection followed by exposure to OVA aerosol.[67]

Sheep has provided a model in which the early and late phase responses to allergen could be examined. There is a natural variability within sheep that in development of an allergic physiological response to inhaled allergen. The response in sheep is characterized by an influx of inflammatory cells into the airways, which includes eosinophils and neutrophils.[68] SC injection of OVA for several weeks was used for sensitization of sheep.[69]

Although, larger animals such as monkey, sheep and horse have been used in asthma models, they are

hard to manipulate and too expensive. Horses naturally develop a respiratory disturbance characterized by acute airway obstruction.[70] Clinically affected horses are typically hyper-reactive to inhaled histamine.[70] An asthmatic model was confirmed in this animals by the presence of serum antibodies and response to aerosol antigen provocation challenge.[70] Horses could be sensitized to OVA by IM injection followed by IT instillation[71] or exposure to OVA aerosol.[72] Table 1 shows a summary of different methods for sensitization of other animals to OVA.

House Dust Mite (HDM)

Inhaled delivery of HDM has been successful in inducing animal model of asthma, possibly because of the intrinsic enzymatic activity of this allergen.[73]

Mice

In some studies BALB/c female mice were exposed to either HDM extract intra-nasally for five consecutive days, followed by 2 days rest, for up to seven consecutive weeks.[74] Exposure to HDM, continuously, leads to severe and persistent airway inflammation. In other studies HDM model for allergic airway inflammation was induced by exposure of female BALB/c mice to HDM extract intra-nasally (25 μg/50 μL) for 5 consecutive days a week over 5 or 7 weeks.[75,76] In another study, male C57Bl/6 mice were immunized with purified HDM intra-peritoneally on day 0 and from day 14-20, the mice were exposed daily to a 30-min aerosol of different concentrations of HDM extract,[77] (Table 1).

Other Animals

Horses were kept together in a low antigenic environment for more than 3 months prior to the baseline measurements and were then stabled in box stalls for 30 days, where they were exposed to hay and barn dust.[72] Inhaled fluticasone propionate and oral prednisolone inhibited the allergen-induced airway hyper responsiveness in animals sensitized with HDM. Monkey asthma models were successfully developed by sensitization with HDM under a short-term protocol (within 7 weeks). These models could be useful for the evaluation of anti-inflammatory drugs for asthma treatment, [78] (Table 1).

Other Agents

To develop animal models of asthma in a short period of time, male monkeys could be sensitized with dinitrophenyl-ascaris suum allergen by IP and IM injection and by IT inhalation. Sensitized animals developed positive intra-dermal skin reaction to ascaris suum allergen. Sensitization elevated allergen-specific IgE levels in serum.[78] The inhalation of ascaris suum using a newly devised apparatus caused a marked asthmatic response with insignificant effects on blood pressure.[35] The monkey provides a model of IgE-mediated acute allergic airway response. These animals demonstrate both skin and respiratory reactivity to the antigen, and develop hyper-reactive airways to histamine and carbacholine.[79] In some protocols, exposures to aspergillosis antigens were used over a period of 2–5 weeks to 10–12 weeks. Most antigens associated with aspergillosis antigens appear to be constituents of the crude extract. Interestingly, in the absence of exogenous adjuvants, potent sensitization to the extract occurred.[80] It is suggested that TNF might be released in the lung following cotton dust exposure, which is associated with the pulmonary inflammatory response. In this manner guinea pigs were exposed to an atmosphere of 33 mg/m^3 cotton dust for up to 6 h. At 3, 6, 7.5, and 24 h, then lungs were isolated and lavaged to assess TNF production.[51] In another study, a significant increase in total serum IgE levels in animals exposed to latex antigens as compared to controls was reported. In this study, latex extract was isolated from sap collected from the rubber plant, *Hevea brasiliensis* was injected IP. in mice, once a week for two weeks.[81] In other investigations mouse was challenged with a mixture of house dust mite, ragweed, and aspergillums species (DRA) allergens to mimic the severe airway inflammation observed in human patients.[82] In a novel mouse model, interaction between recombinant cockroach (r Bla g 2) and dust mite (r Der f 1) allergens in inbred mouse strain was compared to each allergen alone and enhanced airway inflammation and epithelial damage were detected.[83] Different agents for inducing animal model of asthma in various animal spices are summarized in Table 1.

Different measured parameters

Airway hyper-responsiveness (AHR)

AHR is the most important feature of asthma and has been assessed in many experimental models of asthma. In animals AHR could be measured in vivo or in vitro.

In vivo measurement of AHR

Mice

Mice were sensitized and subsequently challenged with OVA and AHR was assessed by Mch challenge and airway resistance was measured using whole body plethysmography.[24,84] The in vivo model of HDM-induced allergic airway changes suggests that AHR is not related to eosinophil influx or allergen specific serum IgE.[77] In vivo AHR was usually examined by measurement of lung resistance using whole-body plethysmograph after inhalation of increment doses of Mch aerosol.[24,25,31,85-87] Airway reactivity could be also assessed by measuring broncho-constriction following IV administration of Mch.[88]

Rat

AHR was estimated by measuring changes in airway resistance response to increasing concentrations of inhaled Mch. Increasing concentrations of Mch aerosol (3.125, 6.25, 12.5, 25 mg/mL) were administered via nebulizer into the head chamber after airway pressure stabilization. Minimum values for airway resistance were measured and AHR was expressed as percentage of change from the baseline value.[26,89] Dose response curves to Mch were also constructed by repeated administration of Mch from 10^{-9} to 10^{-1} M and airway internal luminal area was measured at successive Mch concentrations.[90] In addition in vivo AHR in rat could be examined by IV injection of Mch, 0.0625 mg/kg up to 1 mg/kg at 5 min intervals and measurement of airway resistance using plethysmograph.[38,40] The results indicate that the AHR in the rat reflects that the responsiveness is an intrinsic characteristic of airway smooth muscle.[90]

Guinea Pig

Lung resistance was assessed by plethysmograph, 1 h after histamine aerosol inhalation, upon return to normal breathing patterns.[50]

Dog

In 24 or 35 week-old dog, AHR to MCh was tested 1 and 4 days after ragweed challenge at 28 or 39 weeks of age.[66] Different methods of in vivo AHR measurements in various animals are summarized in Table 2.

In vitro measurement of AHR

Rat

After preparation of tracheal chain and parenchymal strip, cumulative concentrations of Mch was added to organ bath and concentration-response curve was constructed by measuring airway internal luminal area of tracheal stripe. In addition, cumulative concentration curve to isoprenaline was obtained.[91] In another study, lung slice was prepared and dose-response curves to Mch were then constructed by repeated administration of Mch. Airway internal luminal area (Ai) was measured at successive Mch concentrations from 10^{-9} to 10^{-1} M and the effective concentration leading to 50% of the achieved maximal response (EC_{50}) was determined.[90]

Guinea Pig

Specific tracheal chain responsiveness to OVA could be measured in tracheal chain by assessing the proportion of contraction obtained due to 0.1% OVA solution in relation to contraction obtained by 10µM Mch.[92,93]

In several studies tracheal responsiveness to Mch, histamine, and isopernaline were examined using cumulative concentrations of the corresponding agent and determination of EC_{50} and maximum response.[45,92,94-96] Table 2 provides different methods of in vitro AHR measurements in various animals.

Lung Pathology Changes and Remodeling

The pathologic features of fatal asthma including; edema, thickening of the membrane, disruption of the epithelium and inflammatory cell infiltration have been known for long time.[97] Several research groups have developed chronic allergen models in order to reproduce the features of clinical asthma such as goblet cell metaplasia, epithelial hypertrophy, sub epithelial fibrosis and smooth muscle hyperplasia, which together are referred as airway remodeling.[84,98] Remodeling is thought to be a result of repeated exposure to allergen which causes repeated inflammatory events in the airways.[99] The airway remodeling is a characteristic feature of asthma which is thought to contribute to the development of symptoms associated with asthma.

Mice

Extensive mucous plugging occurs in the airways associated with goblet cell hyperplasia. Mucus hyper secretion in the lumen of airways was observed in a mice model of asthma.[100] Hyperplasia and hypertrophy of airway smooth muscle leading to structural changes in the airway wall, increased airway smooth muscle (50-83%), goblet cell hyperplasia/hypertrophy,

Table 2. Airway hyper-responsiveness and lung pathology evaluation in animal model of asthma

Parameters		Animals	Methods	Ref
AHR	**In vivo**	**Mice**	- Inhaled Mch: 3.125 , 6.25, 12.5, 25, 50 mg/mL, 3 min, measuring LR by WBPle	(86)
			- Inhaled Mch aerosl 0.3 mg/mL up to 100 mg/mL (10 sec), measuring LR using Ple	(161)
			- Inhaled Mch 12.5, 25, 50 mg/mL for 5 min with 1 min recovery between subsequent doses, measuring LR using Ple	(24)
			- Inhaled Mch 12.5, 25, 50, 100 mg/mL for 2 min, 6-min monitoring cycle, measuring LR, using Ple	(25)
			- Inhaled Mch 0, 6.25, 12.5, 25, 50, 100 mg/mL PBS, 5 min, measuring LR using Ple	(85)
			Aerosolize Mch (10–40 mg/mL) for 2 min into the chamber, measuring LR using WBPle after 5 min	(28)
			- Mch by double-chamber, measuring LR using WBPle	(84)
			- Inhaled Mch 3, 10, 30 mg/mL, measuring LR using WBPle	(31)
			- Inhaled Mch from 2.5 to 50 mg/mL for 3 min, LR using Ple	(87)
		Rat	- Inhaled Mch aerosol 6.25, 12.5, 25 mg/mL for 2.5 min, measuring LR using Ple	(26, 89)
			- IV Mch 0.0625 mg/kg up to 1 mg/kg at 5 min intervals measuring AR using Ple	(38)
			-IV Mch (0.03, 0.1, 0.3, 1.0 and 3.0 mg/mL in PBS), measuring LR using Ple	(40)
		Guinea pig	- Inhaled histamine 0.125 mg m^{-3} for 15 min, measuring LR using Ple	(50)
		Sheep	- Measuring AR before and 24 h after inhaled carbachol, caused 400% increase in specific LR.	(68)
		Dog	- Inhaled Mch (10^{-3}-10^{-7}), for 5 min, measuring lung resistance use HRCT	(162)
	In vitro	**Rat**	- Tracheal strips preparation, increased muscle tone by 3.10^{-7} M Mch, cumulative dose response curve to isoprenaline, measuring maximal contraction	(91)
			- Preparation of lung-agarose sections (0.5- to 1.0-mm transverse slices), concentration-response curves to Mch (10^{-9} - 10^{-1} M), calculation EC_{50}	(90)
		Guinea pig	- TC preparation, contraction with 10^{-5}mmol/L Mech, measuring EC_{50} using CRC to isoprenaline (10^{-4} -10^{-9} mmol/L), (AR to β-agonist)	(45)
			- TC preparation, Mch (10^{-7} to 10^{-5}mmol/L) every three minmin, measuring EC_{50} and MR using CRC.	(92, 94)
			- TC preparation, TR measuring to 0.1% solution of OVA, measuring contractility response to OVA.	(93)
			- TC preparation; histamine (0.1-320μM) every 7 min, measuring EC_{50} using CRC.	(95)
			- TC preparation; histamine (0.1μM –10 mM) every 2 min, measuring EC_{50} and MR using CRC.	(96)
Lung Pathology		**Rat**	- Lung parenchymal injury, SM thickening	(89)
			eutrophil infiltration, and increased proteomic level in BALF	(138)
		Guinea pig	- Infiltration of eosinophils, epithelial necrosis, edema, smooth muscle hypertrophy, mucosal secretion,	(111-113)
			- Epithelial damage, interstitial expansion, lung congestion, atelectasis, bleeding and epithelial damage	(48, 54)
		Mice	- Hyperplasia and hypertrophy leading to structural changes in the airway wall, smooth muscle cell, contractile function	(11, 14, 101, 102)
			- Increased airway smooth muscle (50-83%), goblet cell hyperplasia/hypertrophy, subepithelial fibrosis, smooth muscle hyperplasia/hypertrophy	(103-105)
			- Increased airway epithelial hypertrophy, goblet cell metaplasia/mucus accumulation	(106)
			- goblet cell hyperplasia, epithelial hypertrophy, and either subepithelial or peribronchiolar fibrosis	(107)
			- inflammatory cell infiltration in the peribronchial and perivascular areas	(108, 109)

AHR: airway hyperresponsiveness; WBPle: whole-body plethysmograph; Ple: plethysmograph; Mch: methacholine; LR: lung resistance; Raw: airways resistance; HRCT: high resolution computed tomography; TR: tracheal responsiveness; OVA: ovalbumin; BALF: broncho-alveolar lavage; TC: tracheal chain;; EC_{50}: effective concentration causing 50% of maximum response (MR); CRC: concentration response curve; AR: Airway reactivity.

sub-epithelial fibrosis, smooth muscle hyperplasia/hypertrophy also were shown in mice model of asthma.[11,14,101-107] Inflammatory cell infiltration in the peribronchial and perivascular areas was also observed in OVA-challenged mice.[108,109]

Rat

In some studies development of inflammation and thickening of the smooth muscle layer was shown in rat model of asthma.[33] Acute lung injury assessed by albumin leakage, neutrophil infiltration, lung histology, and increased proteomic level in BALF were also observed in this animal model of asthma.[110]

Guinea Pig

A characteristic lesion of asthma is excessive production of mucin in the airways. Mechanistic studies of this lesion in guinea pigs have been limited due to lack of mucin gene probes for this species.[111] Infiltration of eosinophils, epithelial necrosis, edema, smooth muscle hypertrophy, mucosal secretion were showed in guinea pig model of asthma.[111-113] In addition, epithelial damage, interstitial expansion, lung congestion, bleeding and epithelial damage were reported in this model.[48,54] Lung pathological changes in different animal models of asthma are summarized in Table 2.

Inflammatory Cells and Mediators

Total and Differential WBC Counts in the Blood and Lung Lavage

Mice

Increased eosinophilia in both blood[81,114] and lung lavage[24,30,34,76,84,89,106,115] were reported in mouse model of asthma.

Rat

Differential WBC, [110] eosinophil, T-cell, and goblet cell counts in blood[39] as well as total WBC[38] and eosinophil[92,116] in lung lavage of rat model of asthma were increased.[110]

Guinea Pig

Total WBC and eosinophil counts in sensitized guinea pigs to OVA were increased.[117,118] In another study, neutrophil infiltration in lung lavage of guinea pigs exposed to an atmosphere cotton dust was shown.[119] Total and differential WBC in blood of OVA sensitized guinea pigs were also observed.[94,112] Total and differential WBC, macrophage, eosinophil, lymphocyte and neutrophil in lung lavage of asthmatic guinea pigs were increased.[33,58,88,112] In Table 3 a summary of total and differential WBC in the blood and lung lavage in different animal models of asthma are presented.

Inflammatory Mediators and Cytokines

Sensitization protocols are key features for inducing animal model of asthma, leading to increased levels of total IgE, in response to allergen.[120] Airways inflammation mediators were found in bronchoalveolar lavage (BAL).[121] BAL fluid (BALF) inflammatory mediators were assessed to test lung inflammation in animal model of asthma.[122]

In an allergic airway inflammation model with antigens, depletion of CD4+ and CD25+ cells, increased lung eosinophilia, increased IL-5 and IL-13, but not IL-10 were shown. Evidence suggests that interleukin-13 has an important role in asthma.[123] Finally, utilization of an IL-13-specific neutralization strategy with soluble IL-13 receptor fusion protein has demonstrated the pivotal role of this cytokine in experimental OVA-induced airway disease.[124] Recently, an important role for type 2 innate lymphoid cells (ILC2) was demonstrated in asthma pathogenesis.[125] These cells were involved in asthma mouse models, including HDM-driven allergic airway inflammation by providing a critical early source of type 2 cytokines, such as IL-5 and IL-13.[126,127] In addition, studies indicate that various immunological factors such as IL-33 play an important role in pathological processes of asthma.[128] Moreover, the forkhead/winged helix transcription factor FOXP3 serve as a master regulator for Treg (regulatory T cell) development and is currently found to be the most specific Treg marker.[129] Preventive effect of Treg cells in allergic patients have been reported by suppressing the activity of Th2 cells and its cytokines.[130] On the other hand, it has been shown that IL-17 secreted by Th17 cells plays an important role in autoimmune disorders and chronic inflammation.[131]

Mice

Anti OVA IgE levels in serum were increased after two weeks in the lung lavage of mice systemically sensitized to OVA and chronically

Table 3. Inflammatory cells and markers in the blood and lung lavage of different animal model of asthma

Sample	Parameters	Animals	Methods	References
Blood	**WBC**	**Mice**	- Eosinophils	(81, 114)
		Rat	- Differential WBC	(110)
			- Eosinophil, T-cell, and goblet cell counts	(39)
		Guinea pig	- Eosinophils	(112)
			- Total and differential WBC in blood	(94)
	Mediators	**Mice**	- TNF-α, NF-κB	(114)
	& Cytokines		- IL-8	(26)
			- IL-2, IL-5, IL-6, IL-10, IL-13, GM-CSF, and IFN- γ	(132)
			-IgE and Plasma corticosterone	(32, 81, 87,
			- IgE	105)
			- NO	(133)
			-IgG1	(32)
		Rat	- TNF-α	(110, 138)
			- IL-8, IL-35, TNF-α, IgE	(40, 56, 139)
		Guinea pig	- Th1/Th2 cytokine (IFN-γ/IL4)	(140)
			- IL-4 and IFN-γ	(51)
			- Serum total protein, PLA2, histamines, and IgE	(117)
			- Serum levels of IL-4 and IFN-γ	(92)
			- IL-4 and IFN-γ levels	(93)
			- Serum levels of endothelin	(52)
			- Serum levels of total NO and nitrite	(55)
Lavage	**WBC**	**Mice**	- Eosinophils	(24, 89, 115)
			- Total and differential WBC in lung lavage	(76, 84)
			- Macrophages, eosinophils, lymphocytes, neutrophils (Cells)	(30, 106)
			- eosinophils, lymphocytes, monocytes	(34)
		Rat	- Eosinophils	(92, 116)
			- Leukocytes	(38)
		Guinea pig	- Total and differential WBC in lung lavage	(58, 88, 112)
			-Macrophages, eosinophils, lymphocytes, neutrophils (Cells)	(33)
	Mediators	**Mice**	- TGF-β	(24)
	&		- Th2 cytokine	(115)
	Cytokines		- Cytokine, and MMP-9	(89)
			- IL-2, IL-5, IL-6, IFN- γ	(28)
			-TGF-β concentrations	(33)
			- IL-4, IL-5 and IFN- γ	(134)
			- IL-4, IL-5, IL-10, IL-13 and TGF-β1	(84)
			- IL-4, IL-13, IFN- γ and TGF-β1	(105)
			- IL-4, IL-5 and IL-13	(76)
			- Histamin, EPO, IgE	(31, 136, 137)
		Rat	- CD4+Foxp3+Tregs, IL-8, IL-35, and TNF-α	(138)
			- IL-4 and IL-13	(38)
			- IL- 4, IL- 5 and IL- 13	(40)
		Guinea pig	- IL-4 and IFN-γ	(77)
			- IL-4	(88)
			- TNF-α protein level	(141)

WBC: white blood cell; NO: nitric oxide; MMP: matrix metalloproteinase; EPO: eosinophil peroxidases

challenged with aerosolized OVA.[88] IL-10 potently suppresses total IgE, and it simultaneously increases IgG4 production in mice model of asthma.[115] Increased TNF-α, NF-κB, NO, IL-8, IL-2, IL-5, IL-6, IL-10, IL-13, GM-CSF, IFN-γ, IgE, IgG1, and plasma corticosterone in blood of asthmatic mice were observed.[26, 32, 34, 81, 87, 105, 114, 132, 133] Change in TGF-β, Th2 cytokines, IgE, MMP-9, IL-2, IL-5, IL-6, IL-10, IL-13, IFN- γ, IL-4, and IL-5 were seen in lung lavage of asthmatic mice.[24, 28, 31, 33, 76, 84, 87, 89, 105, 115, 134, 135] Among the released mediators, histamine from mast cells and eosinophil peroxidases (EPO) are important markers of inflammation in the allergic reactions. Histamine and EPO levels in lung lavage have been reported to increase in OVA-induced asthma in mice.[136, 137] In a study IL-33 levels in lung lavage were significantly increased in both the initial and chronic phase of allergens exposure in mice[128]. In addition, decreased FOXP3 genes expression but increased IL-17 gene expression in mice sensitized to OVA was demonstrated.[129]

Rat

Total protein levels in the BALF, and intra-alveolar edema increased in rat model of asthma.[110] Increased levels of IL-8, IL-35, TNF-α and IgE were also seen in blood of sensitized rat.[40, 56, 110, 138, 139] In addition CD4+Foxp3+Tregs, IL-4, IL-5, IL-13, IL-8, IL-35, and TNF-α increased in lung lavage of asthmatic rat.[40,138]

Guinea Pig

Guinea pigs have been used as a model to investigate the course of bronchopulmonary inflammation following immunization with toluene diisocyanate.[140] There was a marked increase in TNF in BALF of asthmatic guinea pigs.[117] The role of Th2 cytokine pathways in the pathogenesis of goblet cell hyperplasia in asthmatic airway epithelium in sensitized guinea pigs was reported.[51] Serum levels of total protein, PLA2, histamines, IgE, IL-4, IFN-γ, endothelin, total NO and nitrite increased in guinea pig model of asthma.[52, 55, 93, 117] IL-4, IFN-γ, TNF-α, and protein levels of BAL increased in sensitized guinea pigs.[77,141] Inflammatory mediators and cytokines changes in the blood and lung lavage in different animals model of asthma are presented in Table 3.

Lung Tissue Inflammatory Gene Expression

TaqMan low density array was performed for lung tissue inflammatory gene expression Involved genes in asthma were studied by their differential expression in microarray studies of several asthma models.[76,105] Reverse transcription (RT) of RNA was also carried out for target (iNOS, TGF-β1 and TNF-α) genes.[34]

Immunohistochemistry

Mouse models of asthma are capable of recapitulating some components of asthma and have been used to look at both IL-13 and TGF-b1 pathways, which use STAT6 and SMAD2 signaling molecules, respectively. Sections of lung were immunestained using antibodies against a-smooth muscle actin (a-SMA), phospho-SMAD2 (pSMAD2), phospho-STAT6 (pSTAT6) and phosphor-Tyr705 STAT3.[82,105] In another study immunochemistry was done in lung sections for iNOS and eNOS, because increased NO production is another important characteristic feature of asthma.[84,87] Recent studies have indicated that increased mass of airway smooth muscle cells (ASMCs) plays a critical role in the histopathological characteristics of airway remodeling in asthma. Thus immunohistochemistry was performed using the monoclonal mouse antibody to α-smooth muscle actin (α-SMA).[109] The level of Notch1 was significantly higher in asthmatic mice and Notch1 signal may play an important role in the pathogenesis of asthma by its involvement in Th1/Th2 differentiation. Immunohistochemistry was carried out using primary antibodies, goat polyclonal anti-Notch1 and rabbit polyclonal anti-Notch2.[142] In another study, lung sections were examined for cells producing IFN-γ, IL-4, IL-5, IL-10, and IL-13 by immunohistochemistry anticytokine antibodies.[81]

Transgenic Model of Asthma

In recent years, transgenic animals are used to analyze pathophysiologic characteristics of allergic asthma such as allergic airway inflammation and airway remodeling, which provides important information for the human disease.[143,144] More specifically asthma is difficult to model, since we do not understand its exact cause. It is well known that exposure to allergens, is the most common stimuli in asthmatic patients. Therefore, systemic sensitization and subsequent airway challenge with OVA may lead to a phenotype similar to these aspects of asthma including lung inflammation eosinophilia and hyperreactivity.[145] With these models, it is possible

to switch off, suppress or upregulate a single molecular pathway to understand the importance of this pathway in the development of the asthmatic phenotype.[146]

Mice are the most commonly used species because they are easier to reproduce, maintain, and use. Rats have also some more advantages than mice for studies on inhaled agents, but are not fully suitable for transgenic or knockout models.[147] In addition, there is a vast diversity of reagents for determination of antibodies and cytokines, as well as transgenic strains, which are used to study the mechanisms of diseases.[147] Expression of particular cytokines or growth factors under the control of a lung-specific promoter has determined roles for these molecules in some of the pathophysiological characteristics of asthma.[148] These studies have indicated the significance of the cytokines IL-4, IL-5 and IL-13, most of which are indicators of Th-2 responses in pulmonary inflammation.[144,145] Moreover, among various immunological factors, IL-33 showed to be important in mediating the inflammatory process in asthma. This cytokine is significantly enhanced during both the early and chronic phases of exposure, because blockage of the pathway weakens airway pathology changes. Also, thymic stromal lymphopoietin (TSLP) contributed importantly, whereas IL-25 and IL-1 involved slightly in the process of airway inflammation in asthma.[128] Direct targeting of growth factor (TGF)-β and vascular endothelial growth factor (VEGF) indicate phenotypic similarities with asthmatics. Lung-targeted VEGF resulted in an asthmatic phenotype determined by inflammation, parenchymal and vascular remodeling, edema, mucus metaplasia, myocyte hyperplasia, and AHR.[148] The role of IFN-inducible protein10 (IP-10) in allergic airway disease was also studied by evaluating the expression of IP-10 in a murine model of asthma and examining the effects of over-expression and deletion of IP-10 in this model using IP-10-transgenic and IP-10-deficient mice. Mice deficient in IP-10 demonstrated a significant reduction in Th2-type allergic airway inflammation compared to wild-type controls. Results demonstrated that IP-10, a Th1-type chemokine, is up-regulated in allergic pulmonary inflammation and that this contributes to the airway hyper-reactivity and Th2-type inflammation seen in this model of asthma.[149] Models with over-expression of the Th2-specific transcription factor GATA3 or the Th17-transcription factor RORγt provide methods for studying the role of Th2 and Th17 immune response in allergic airway disease.[150,151] In another study, Mice deficient in $Il1r1^{-/-}$ (i.e. deficient in the receptors for IL-1α and IL-1β), Il17rb−/− (i.e. IL-25 receptor deficient), $ST2^{-/-}$ (i.e. IL-33 receptor deficient) and TSLPR−/− (TSLP receptor deficient) were exposed to a mixture of allergen 3 times a week, for up to 8 weeks under isoflurane inhalation anesthesia. Mice deficient in IL-33 receptor and TSLP receptor showed significant decline in airway inflammation, IgE antibody levels, and AHR. In contrast, mice deficient in IL-25 receptor or IL-1 receptor indicated little differences in comparison with wild-type animals.[128] Also, over-expression of TGF-β in the epithelium of a mouse model induced a primary wave of epithelial apoptosis that decreased with continued expression of TGF-β. Prominent inflammation was also noted, as well as an airway and parenchymal fibrotic response characterized by increased collagen deposition. Moreover, there was a significant increase in accumulation of myofibroblasts and myocytes.[148] In another study, an allergen-naive double transgenic mouse model showed increased IL-5 systemically from mature T cells and eotaxin-2 locally from lung epithelial cells. Results demonstrated that these mice developed several pulmonary pathological feature of severe asthma.[152]

Although, the transgenic model provides reliable models for the preclinical approval of therapy in allergic asthma, but further studies are needed to confirm that this cytokine or protein has the capacity to cause the phenotype.[153]

When designing animal models of asthma, it is important to clearly decide which aspects of lung structure and/or function associated with asthma needs to be addressed. Development of a representative model will therefore have to take into account knowledge of animal biology, the method of induction of asthma and proposed outcomes as well as the characteristics of human asthma that need to be modeled (Figure 1). The present review, provides information regarding induction of animal model of asthma in different animals, various methods used for this purpose, and measured parameters, which would help investigators to select the appropriate animal, method and parameters depending on their study design.

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