INVITED REVIEW

Animal models of asthma

Tunç Akkoç[1]

ABSTRACT: Allergic disease such as asthma, rhinitis, and eczema are increasing prevelance and affect up to 15% of population in Westernized countries. Among them, asthma is a chronic inflammatory disease of airways and the underlying physiological and immunological processes are not fully understood. Mouse models of asthma dupicates many features of human asthma, including airway hyperreactivity, andairway inflammation. Therefore, relevant models for asthma are important to understand the mechanism of disease and therapeutical approach. In this article, basicly various animal models of asthma and some therapeutic approaches are disscussed.

KEY WORDS: Asthma,Mouse, Ovalbumin, Regulatory cells

AFFILIATIONS
[1]Marmara University, Pediatric Allergy and Immunology Department, İstanbul, Türkiye

CORRESPONDENCE
Tunç Akkoç
E-mail:
tuncakkoc@yahoo.com

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INTRODUCTION

The prevelance of allergic diseases such as allergic asthma, allergic rhinitis, atopic dermatitis and food allergy are rapidly increasing mostly in Westernized countries, with the prevelance over %30 in childhood (1-2). Human allergic asthma is a chronic inflammatory disorder of the airways and is chracterized by airway inflammation, persistent airway hyperresponsiveness (AHR) and intermittent, reversible airways obstruction (3). Additional histopathological features of asthma are structural changes in airways including subepithelial and airway wall fibrosis, goblet cell hyperplasia/metaplasia, smooth muscle thickening and increased vascularity, that are shown not to be reversed by corticosteroids (4-5). These structural changes referred as 'airway remodelling' and result with repeated exposure to allergen, that lead to chronic inflammation in the airways (6).

Although, today there are several therapeutic and preventive approaches disscussing for allergic asthma, there is still important part left to be understood. Because of the ethical reasons, clinal studies with allergic individuals limited and experimental murine models became more important for drug treatment studies.

Animal models, especially mouse models, have been developed for almost all allergic disease as asthma (7), allergic rhinitis (8), food allergy and anaphylaxis (9), atopic dermatitis (10), and allergic conjunctivitis (11). These murine models are important in order to examine the mechanism of the disease, the activiy of a variety of genes and cellular patways, and to predict the safety of new drugs or chemicals before being used in clinical studies (12). As a result, animal models have been developed to study pathogenesis of the diseas, including genetic factors, to define the pathogenic pathways and suggest new therapeutic views (13). This review focuses on the murine models of asthma and disscusses current therapeutic approaches carried on mouse models of asthma.

Immune response to allergens

In the case of immune tolerans cannot be established to certain allergens such as, aeorallergens, foods and insect venom, leads to induction of type I hypersensitivity reactions. 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 challenge with infections and bacteria, (14) influence the type of immune response.

The initial event responsible for the development of allergic disease is the generation of allergen-specific $CD4^+$T helper cells. Depending on the stimulus, naive T cells can differentiate into Th1, Th2, Th17 or recently suggested Th9 effector cells. Based on these T-cell subsets can promote different types of inflammatory responses. The current view is that with the IL-4 stimulation in an appropriate conditions, naive T cells easly differentiate into T helper (Th) 2 cells (15-16). Once Th2 response is established, further allergic immune response may establihed in two main phas-

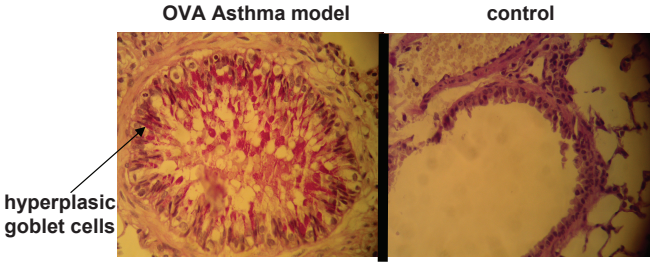

FIGURE 1. PAS stained lung sections of all groups. (40x)

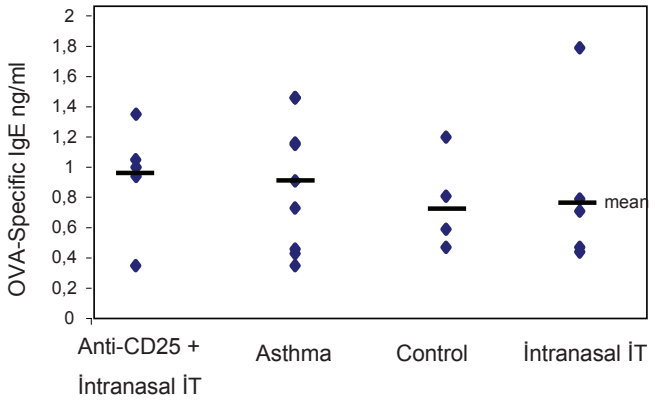

FIGURE 2. OVA-specific IgE levels.

es: first sensitization and development of memory and later followed by effector phase and tissue injury. In sensitization phase, $CD4^{+}Th2$ cells secrete IL-4, IL-5 and IL-13 and mediate several regulatory and effector functions. These cytokines induce class switching of antibody isotypes to the e heavy chain for IgE antibody production by B cells, development and recruitment of eosinophils, production of mucus and contraction of smooth muscles (15-17). Later, this allergen specific IgE, binds to high affinity receptor for IgE (FceRI receptors) on the surface of mast cells and basophils. These series of activation lead to the sensitization of the patients to a specific allergen. In the final phase, upon the re-exposure to sensitized allergens, effector cells are activated and tissue injury happens. Here, the degranulation of basophils and mast cells by IgE mediated by crosslinking of receptors that is the crucial event in the type I hypersensitivity, which may lead to chronic allergic inflammation. All these events require allergen specific T cell activation in allergic individuals and for healthy ones, peripheral T cell tolerans prevents formation of atopic immunopathology.

Animals used in allergic asthma models

Experimental studies revealed that, there is wide variety of animal models of asthma in different specicies. Mice, rats, guinea pigs, dogs, sheep, monkeys and horses have been employed to study the inflammatory processes and alternations in airway function (18-20).

Within these animals, mice are the most common species studied in animal models of asthma. We will disscuss mouse models of asthma later in this review.

Although a majority of studies of allergic airway disease are carried out in the mouse, the guinea pig initially was utilized as an animal model of pulmonary hypersensitivity and AHR for many decades (21). Studies with guinea pig showed increased IgG1 and IgE in response to allergen and hyperreactivity reaction due to allergen sensitization. Model studies showed, allergen sensitization require allergen inhalation. One of the benefit of the model is the lung surves as the primary target organ of type I hypersensitivity reactions to allergen. Further immediate and late phase reactions are also seen in carried asthma model. Another advantage of the asthma model with guinea pigs is the rich eosinophilic and neutrophilic pulmonary infiltration. On the other hand, one of the major disadvantage to the guinea pig is the predominance of IgG1 rather than IgE as the major anaphylactic antibody (22).

In several studies, investigators have been used another small rodent, rat, for allergic animal models. Allergen specific IgE production is predominant and plays crucial role in anaphylaxis. Once, asthma model with rat developed, there is long-lasting airway hyperreactivity. Also immediate- and late-phase airway responses established in the succesfull asthma model. In the comparision with guinea pig asthma model, sensitization requries injection of allergen, rather than administration via the inhalation route (23-24).

The rabbit elicit an asthma model and lung is the target organ for anaphylactic response as humans. Moreover, both immediate- and late-phase airway responses seen with the increased IgE which is anaphylactic antibody.

Although, larger animals such as monkey, sheep and horses have been used in asthma models, they are hard to handle and too expensive in order to use on regular basis.

Mouse models of asthma

Mice are the most common species studied in animal models of asthma. In principle, mice are systemically sensitized to allergen with alum as an adjuvant via intraperitoneal injection and challenged to allergen via the airways. The nature of acute inflammatory mouse model directly influenced by the genetic background of the mice, the allergen, the sensitization and challenge protocol (6).

Genetic background of mice

In laboratory conditions, it is aviable to get various inbred mouse strains. However, it is inappropriate to develope asthma model with all these mouse strains. Based on the level of allergen-specific IgE/IgG1 production and the degree of airway inflammation following repeated airway allergen challenges, high- and low- responder mouse strains were identified. Each different mouse strains show different response pattern following immunization to allergens. There are difference in the ability to induce allergic inflammation and AHR within those mouse strains. A/J and AKR/J mice bare high levels of allergen-induced AHR and reactivity to metacholine (25) while, C3H/HeJ and DBA/2 mice resistant to the development of allergen-induced AHR (26).

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. Ovalbumin, Bet v 1) and goat anti-mouse IgD-stimulation, whereas C57BL/6 mice is characterized as low-IgE responder animal (27). Supportingly, several experimental

mouse models with BALB/c strain, showed well developed Ovalbumin (OVA) induced allergic immune response with allergen-specific IgE, AHR and eosinophilic airway inflammation (4). On the other side, C57BL/6 mice exhibit Th1-dominant immune response compared to BALB/c mice.

Allergen

In order to perform murine asthma model, different types of allergens may be used. Among them, OVA derived from chicken egg is a frequently used allergen that induce robust, allergic pulmonary inflammation in mice (28). There are some advantage to use OVA in models such as considerably inexpensive, can be highly purified, the immunodominant epitopes have been well characterized, and recombinant peptides have been generated (29).

The way of allergen administration is important in develeoping asthma model without induction of tolerance. Especially, repeated inhalation or sublingual administration of OVA induce tolerance instead of sensitization. For priming allergen sensitization, allergen should be given combined with adjuvant and via intraperitoneal injection (30). Following sensitization, a series of inhaled or intranasal challenges are administered to elicit allergic response. OVA-induced allergic airway models may not represent the same conditions experienced by asthmatics where allergen exposure may be more frequent and much longer periods of time (28). However, OVA is seldom implicated in human asthma, and other gruops have used alternative allergen that have greater clinical relevance, for example house dust mite (HDM) and cockroach extracts (31-32).

Sensitization and challenge protocols,

Mice do not spontaneously develop AHR or allergic airway inflammation. There are many different sensitization and challenge protocols exists. Due to experimental approach, acute or chronic asthma models may be developed. Acute sensitization protocols usually requrie multiple systemic administration of allergen in the presence of an adjuvant. Aluminium hydroxide (ALOH3) is one of the best choise for adjuvant in case, it promote the development of the Th2 immune response when it is exposed to antigen. Although there are adjuvant-free protocols exist, they usually require a greater number of exposures to achive suitable sensitization (33). Both OVA and HDM can be used as an antigen to induce pulmoner inflammation. If OVA administered via inhalation instead of systemic delivery, tolerans can develop. However, inhaled delivery of HDM seems to be more successfull in developing allergy model because of intrinsic enzymatic activity of this allergen. Extracts or purified protein derived from potent human allergens including cockroach, ragweed, or fungi have been increasingly used as allergens in mice and other species (34-36). After sensitization period (usually 14-21 days), allergen challenge via airways will be carried out for several days. The administration of allergen through airways may be applied by nebulization, intratracheal (i.t) or intranasal (i.n.) instillation (4). With these sensitization and challenge protocols mice develop key features of clinical asthma, as increased levels of allergen specific and total IgE, inflammatory cell infiltration to airways which referred as airway inflammation, goblet cell hyperplasia, , epithelial hypertrophy, AHR to specific sensitized allergens or metacholine, early- and late-phase bronchoconstriction in response to allergen challenge (37). (Fig. 1)

Although acute allergen challenge model develops many aspects of human asthma, there are limitations to these models in comparision with asthmatics. For this, several research groups have developed chronic allergen challenge models in order to reproduce more of the features of clinical asthma, such as goblet cell metaplasia, epithelial hypertrophy, subepithelial fibrosis and limites smooth muscle hyperlasia which together refferred as airway remodelling and persistent AHR (4-33). In order to develop chronic asthma model, low levels of allergen should be repeated exposure to the airways to for periods up to 12 weeks. Mostly in an experimental models, OVA has been used as an allergen (38-40) and in some experimental models enviromentally relevant antigens such as, HDM extract or grass polen have been used to develop chronic allegen challenge asthma model (31-41).

Regulatory T cells in murine models in allergic inflammation

T cells with the capacity of suppressing the unwanted immune response were first described in the beginning of 1970s (42). Since mid-1990s, the notion of the T-cell-mediated immune suppression has been strongly explored. T cells with suppressive ability have been described in several systems, and their biology has been subject of intensive investigation. Although recent advances of T cells controlling immune responses via cell-cell interactions and/or the production of cytokines is currently well described, many aspects of the mechanisms through which suppressor cells exert their effects are currently being elucidated (43-45).

Type-1 T Regulatory cells

Type-1 Regulatory (Tr1) cells, also known as inducible Treg, are defined by their ability to produce high levels of IL-10 and transforming growth factor-b (TGF-b) (43-46). Antigen-specific Tr1 cells arise *in vivo,* but may also differentiate from naïve $CD4^+$ T cells. IL-10 and IFN-a have been described to induce the generation of IL-10-producing Tr1 cells from naïve $CD4^+$ T cells activated through T cell receptor and CD28 (47). In order to inhibit Th1 or Th2 differentiation by using anti-IL-12 and anti-IL-4 Abs, together with a combination of dexamethasone and the active form of vitamin D3 , were shown to induce human and naïve $CD4^+$ T cells to differentiate into large numbers of IL-10-producing Tr1 cells in vitro[48]. In addition, immature DCs and DCs treated with IL-10 or IFN-a were shown to induce naïve $CD4^+$ T cells to differentiate into IL-10-producing Tr1 cells (47-49-50). Activation of inducible costimulator (ICOS) on $CD4^+$ T cells by ICOS ligand (ICOSL) enhances differentiation into IL-10-producing Tr1 cells (51-53). During the early course of allergen-specific immunotheraphy (SIT), IL-10- and/or TGF-b-producing Tr1 cells in humans are propagated in vivo, which demonstrates that Tr1 cells are induced by high and increasing dose of allergens (44-54-55). IL-10 that is produced and progressively secreted during allergen-SIT appears to counter-regulate synthesis of allergen-specific IgE and IgG4 (46). IL-10 potently suppresses both total and allergen-spesific IgE, and it stimultaneously increases IgG4 production (56). In control of Th2 immune response against naturally exposed harmless environmental antigens is mediated by Tr1 cell (57). In contrast to several Treg related suppressor factors, OX40L

has an important role in the negative regulation of the generation and function of IL-10-producing Tr1 cells (58).

$CD4^+CD25^+$ Treg Cells

The naturally occurring $CD4^+$ Treg cells, constitute approximately 10% of peripheral $CD4^+$ T cells in normal indivudals, and characteristically express CD25 (the interleukin-2 (IL-2) receptor a chain, that is component of the high-affinity IL-2 receptor)[59]. $CD4^+$ $CD25^+$ Treg cells play major role in maintaining immunological self-tolerance and immune homeostasis. Depletion of this population leads to a variety of autoimmune inflammatory disease such as arthritis and diabetes.

The transcription factor forkhead box p3 (Foxp3) is successfully expressed by $CD4^+CD25^+$ Treg cells and essential for functioning regulatory activity. Foxp3 was originally indentified as the gene product affected in lethal X-linked recessive lymphoproliferative disease in mice and human (60). Mutations in the gene encoding Foxp3 leads lymphoproliferative disease of the scurfy mouse. Male mice with Foxp3 deficiency die about third week of age(61-62) Foxp3 deficient mice also experience allergic dysregulation(63). Adoptive transfer of $CD4^+CD25^+$ Treg cells rescues scurfy mice from disease, and Foxp3-transduced $CD4^+CD25^-$ T cells suppressed wasting and colitis induced by transfer of $CD4^+CD25^-$ T cells into RAG-deficient mice (61-64). Foxp3 mutations also results homologous autoimmune lymphoproliferative disorder in human subjects, termed immune dysregulation polyendocrinopathy enteropathy-X-linked (IPEX) syndrome and X-linked autoimmunity-allergic disregulation syndrome (XLAAD) (65-67). Males represent with neonatal autoimmune type 1 diabetes with islet cell destruction by infiltrating T cells. Another prominent feature of IPEX/XLAAD is severe allergic inflammation with eczema and food allergy. The IgE levels can be dramatically increased with intense peripheral eosinophilia (66-68).

Numerous additional evidence support the role of Foxp3 in Treg cells generation and function. Foxp3-transduced T cells exhibited impaired proliferation and production of cytokines including IL-2 and IL-10 upon TCR stimulation, up-regulated the expression of regulatory T cell-associated molecules such as CD25 and CTLA-4 and suppressed in vitro proliferation of other T cells in a cell-cell contact-dependent manner (60-70). Foxp3 negatively regulates the gene encoding IL-2 and enhances CD25 expression and other Treg cell-associated molecules (60-71-72) Mice genetically deficient in IL-2, CD25, or CD122 (the IL-2 receptor β chain) and humans with genetic deficiency of CD25 have both reduced numbers and impaired function of $Foxp3^+$ Treg cells. This leads to severe autoimmune inflammatory disease (69-73-74). Foxp3 binds to other transcription factors such as NFAT (nuclear factor of activated T cells) and AML1 (acute leukemia-a)/Runx1 (Runt-related transcription factor 1) and potentially interacts with activator protein 1 and nuclear factor kB (75). The interaction of Foxp3 with NFAT leads the expression of CTLA-4 and CD25 (76). Recently, thymic production of $Foxp3^+$ Treg cells were analyzed from birth to 10 years of age in humans. The study suggest that from birth to 10 years of age, the thymic production of $Foxp3^+$ Treg cells is stable and correlates with T-lymphopoiesis. However, there is no correlation between thymic and peripheral Foxp3 levels(77). Taken together Foxp3 is a crucial regulatory gene for the development and function of $CD4^+CD25^+$ regulatory T cells, and can be used as marker for Treg cells. Furthermore, Treg cells de novo produced from normal naive T cells by Foxp3 transduction can be instrumental for treatment of autoimmune/inflammatory diseases that require downregulation of hyperactive immune responses.

Downregulated interleukin-7 receptor (CD127) in Treg cells distinguishes Treg cells from activated T cells, facilitating both Treg-cell purification and their functional characterization in human diseases (78). CD127 has been suggested as a marker, which might distinguish peripheral $Foxp3^+$ Treg cells from activated $Foxp3^+$ nonregulatory T cells(79).

Animal model studies have shown that, naturally occurring $CD4^+CD25^+$ Treg cells can control allergic airway disease. Anti-CD25-mediated Treg cell depletion before house dust mite treatment increased several features of the allergic disease (AHR, eosinophilia, and IgE), which was concomitant with elevated Th2 cytokine production(80). In this study, Treg cell-depleted mice revealed increased numbers of pulmonary MDCs with elevated expression of MHCII, CD80 and CD86. In addition Treg cell-depleted mice have capability to stimulate proliferation of T cells and Th2 type cytokine production with a reduced IL-2 expression. Furthermore, transfer of $CD4^+CD25^+$ T regulatory cells to sensitized mice prevents the features of allergic airway disease *in vivo* (81). This downregulation of eosinophils, Th2 recruitment, AHR and Th2 cytokine production paralleled with concominant increase in pulmonary IL-10 production. These studies highligth that $CD4^+CD25^+$ Treg cells may be used before allergic sensitization to control the development of allergic disease.

In an allergic airway inflammation model with Der p 1 antigen showed that depletion of $CD4^+CD25^+$ $Foxp3^+$ Treg cells exacerbates lung eosinophilia, increased draining mediastinal lymph node IL-5 and IL-13, but not IL-10 secretion(82). Transfer of $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells from naïve mice reverses allergic inflammation with decreasing IL-5 and IL-13 secretion. In parallel experiment increased IL-10 secretion from regional lymph nodes was observered. In a recent study form our group, we showed that in-vivo CD25 neutralization do not decrease OVA-specific IgE in intranasal OVA immunotheraphy group. (Fig.2, Unpublished data)

One of the mechanisms to induce mucosal tolerance is to increase antigen-spesific Treg cells. Using birch pollen allergen intranasally (Bet v 1) before (prophylaxis) or after sensitization (treatment) resulted in increased Foxp3 mRNA expression in $CD4^+$T cells with IL-10 and TGF-β secretion. A prolonged effect was observed 1 year after immunotheraphy. Long-term efficacy of specific tolerance seems to be associated with the presence of $Foxp3^+$ T cells (83).

It was previously reported that high levels of the soluble form of the IL-6R (sIL-6R) stimulates $CD4^+CD25^+$ Treg cell suppressive function in the airways of asthmatic subjects (84). Blockade of IL-6 *in vivo* induced local expansion of $CD4^+CD25^+Foxp3^+$ lung Treg cells with increased immunosuppressive capacities (85). Again, blockade of IL-6 led to a significant decrease in inflammatory cells by an apoptosis-independent mechanism. In another study, local treatment with anti-IL-6R antibodies that also block signaling via the mem-

Astımda hayvan modelleri

ÖZET: Astım, allerjik rinit, ve ekzema gibi allerjik hastalıların prevelansı özellikle batılı ülkelerde popülasyonun yaklaşık %15'ini etkileyecek şekilde artmaktadır. Bu hastalıklar arasında astım havayollarının inflamatuar bir hastalığıdır ve hastalığın altında yatan fizyolojik ve immunolojik mekanizmalar halen tam olarak açıklanamamıştır. Farelede geliştirilen astım modeli insan astımına çok benzemektedir. Bu yüzden geliştirilen uygun modellerde hem hastalık mekanizması hakkında hem de tedavi yakalşımları hakkında önemli veriler elde edilebilmektedir. Bu derlemede, astım için geliştirilen hayvan modelleri ve tedavi yaklaşımları tartışılmıştır

ANAHTAR KELİMELER: Astım, Fare, Ovalbumin, Regülatör hücreler

brane-bound IL-6R (mIL-6R), which led to decreased signal transducers and activators of transcription (STAT)-3 but not STAT-1 phosphorylation in the lung of treated mice. Moreover, this treatment induces apoptosis of the cells present in the airways of OVA-treated mice as well as apoptosis of lung CD4+ effector T cells. Blockade of mIL-6R signaling leads to cell death of lung effector T cells by activating Treg cells in experimental model of asthma(86). These recent data suggest that local targeting of IL-6R signaling upregulates Th2 cell death in allergic airways via Treg cells.

Sublingual administration of two different tricylated pseudodipeptidic molecules modulate Th1/Treg polarization. While both OM-197-MP-AC and OM-294-BA-MP polarize naïve T cells toward the Th1 type with IFN-g production, only OM-294-BA-MP induces IL-10 gene expression on $CD4^+$ naïve T cells (87). In this study, sublingual administration of OM-294-BA-MP with antigen enhances tolerans in OVA sensitized BALB/c with preventing both airways hyperresponsiveness and lung inflammation. In another study, adjuvants stimulating IL-10 gene expression by human or murine immune cells are tested sublingually in BALB/c mice sensitized to OVA. A combination of 1,25-dihydroxyvitamin D3 plus dexamethasone (VitD3/Dex) as well as Lactobacillus plantarum induce IL-10 production by human and murine DCs. Following stimulation with VitD3/Dex-pretreated DCs, CD4+ naïve T cells exhibit a $Foxp3^+$Treg cells(88).

CONCLUSION

Animal models of allergic disease such as, AR, AD, food allergy, allergic conjunctivitis and allergic asthma are valuable models in laboratory studies. Although those models cannot carry all clinical features itself, they are important in order to understand entire mechanisms of the disease and therapeutic approaches. Especially mouse models are being developed to model asthma exacerbations, and investigators are using acute and chronic allergen challenge in their investigations.

Animal models which are more closely reflect asthma, and use of human system will boarden our knowledge of the disease and help identify and evaluate new therapeutic targets.

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