Animal Models of Allergic Diseases

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Abstract: Allergic diseases have great impact on the quality of life of both people and domestic animals. They are increasing in prevalence in both animals and humans, possibly due to the changed lifestyle conditions and the decreased exposure to beneficial microorganisms. Dogs, in particular, suffer from environmental skin allergies and develop a clinical presentation which is very similar to the one of children with eczema. Thus, dogs are a very useful species to improve our understanding on the mechanisms involved in people’s allergies and a natural model to study eczema. Animal models are frequently used to elucidate mechanisms of disease and to control for confounding factors which are present in studies with patients with spontaneously occurring disease and to test new therapies that can be beneficial in both species. It has been found that drugs useful in one species can also have benefits in other species highlighting the importance of a comprehensive understanding of diseases across species and the value of comparative studies. The purpose of the current article is to review allergic diseases across species and to focus on how these diseases compare to the counterpart in people.

Keywords: atopic dermatitis; food allergy; asthma; dogs; humans; mice
1. Introduction

Several types of allergic diseases affect the skin in both humans and animals. One of the most common is atopic dermatitis (AD), also referred to as eczema in human medicine. Atopic disease is genetically inherited in all species and results from complex interactions between genetic and environmental factors. In a subset of patients, AD is linked to allergies. In people, the first manifestation of atopic disease is cutaneous and is frequently first triggered by food allergens. The disease is chronic and relapsing in nature with acute exacerbations frequently associated with allergen exposure. Many studies have shown evidence of various mutations affecting skin barrier function highlighting the fact that sensitization occurs first through defective skin. Due to the increased permeability of the skin, allergens are more readily absorbed and this increased exposure leads to the development of allergic sensitization. As the disease progresses, sensitizations may develop against a variety of environmental allergens and not just foods. Some patients appear to progress from the cutaneous phase to respiratory signs such as asthma as part of the “atopic march”, although debate still exists in human medicine regarding the existence of this phenomenon. Atopic disease has become very common in industrialized countries possibly due to changes in lifestyle and diet and much effort has been devoted to identifying the causes and mechanisms to either prevent or minimize this epidemic of allergies. For this reason many animal models have been studied for both environmental and food allergies. Many similarities, yet some differences exist in the manifestation of AD across species. Some of these differences can be leveraged to improve our understanding regarding the mechanisms involved in the pathogenesis of this complex disease and, in the future, to improve our understanding on why some individuals have multiple manifestations of atopic disease and some only have one clinical sign. In this review, we will discuss the various models by focusing on the primary manifestation of allergy that they were attempting to reproduce and study.

2. Atopic Dermatitis: Comparative Observations

With this term in veterinary medicine, we refer to a recurrent chronic pruritic dermatitis with a characteristic distribution and most often associated with allergen specific IgE against environmental allergens [1]. People also suffer from AD. The term eczema is frequently used interchangeably with AD in human medicine while it has been abandoned in veterinary medicine. Eczema has become quite common in children in westernized countries and it is estimated to affect approximately 40% of preschool children in the USA and the UK [2,3]. This increase has a major impact both medically and financially and it has been referred to as an epidemic of allergies.

The clinical distribution of AD is very similar between children and dogs. Lesions are erythematous, pruritic and prone to be secondarily infected with Staphylococcus spp. Areas that are typically affected are folds and flexural surfaces such as the antebrachial area, axillary, and groin area. The perioral and periorcular regions, hands and feet are also commonly involved.

In both species, AD is frequently but not always linked to an allergic sensitization. There is indeed a subset of patients in both humans and dogs in which the disease is not linked to allergies. This form of AD is called “intrinsic” or “not allergic” to differentiate it from the more classic “extrinsic” or “allergic” form of the disease in which allergen specific IgE are detectable [4–6]. Approximately 10%
of people with AD are affected by the “intrinsic” form. Although the intrinsic form has been considered by some as a step toward the development of extrinsic form, it is becoming increasingly clear that it may actually represent a different subtype [5]. No difference in severity of disease is typically observed between the two subsets of patients although different frequency in distribution of lesions has been described [7]. The intrinsic form tends to develop in older patients and filaggrin mutations are not a characteristic of this form [8]. Studies investigating skin barrier function in patients diagnosed with intrinsic AD have shown that normal function and sensory reactivity to external stimuli is retained in these individuals, which is different from patients with the extrinsic form [9]. Immunologically, lower expression of T helper (Th)2 cytokines such as Interleukin (IL)-4, 5, and 13 are found in affected individuals. The same distinction has been proposed in the dog and the term atopic-like dermatitis” has been proposed for individuals in which no detectable IgE can be found. Multiple immune dysregulations have been reported in AD. It is generally accepted that AD may in part be due to defective immune tolerance [10]. Regulatory mechanisms are necessary to maintain peripheral tolerance by the immune system [11]. Regulatory T cells (Tregs) are a diverse subset of T cells capable of dampening inflammatory cell responses [12,13]. Several studies have shown that Tregs are dysfunctional in atopic patients [5,14] leading to aberrant immune response after allergen exposure. Other immune dysregulations described in allergic skin diseases have highlighted the role of basophils [15] and mast cells [16]. Interestingly, no increased skin mast cells’ releasability [17] and no increased circulating levels of histamine have been reported in human AD patients [18]. Additionally, the usefulness of antihistamines H1 receptor blockers is limited [12]. More interest now exists in the role of H4 receptors and other mast cell products such as tryptase [19,20]. In particular, the histamine H4 receptor has been demonstrated to mediate inflammation and Th2-dependent inflammation and is a potential target for future therapies [21–24].

In dogs with AD, studies examining number of mast cells and the histamine release between normal and atopic dogs showed no differences or even a lower mast cell density in lesional and non-lesional atopic skin compared to healthy control skin [25]. In addition, studies evaluating serum histamine concentrations revealed that levels are similar [26] or lower [27] in dogs with AD compared to healthy controls, thus the role of histamine is still under debate. Interestingly, the few studies evaluating the effect of antihistamines, including H4 blockers, have failed to report a beneficial effect [28,29]. In a subset of human patients with AD, genetically inherited skin barrier defects are demonstrable and further skin barrier impairment occurs once sensitization develops and inflammation is triggered [30]. Thus, skin barrier impairment can be both primary and secondary and it plays some role in all patients at some point during the course of the disease. One of the most documented genetically inherited mutations is in the filaggrin gene. Filaggrin is very important for proper keratinization (the process of differentiation of cutaneous epithelial cells). Mutations in the filaggrin gene have been extensively studied and reported in people with AD to the point that null mutations in this gene, leading to a decrease expression of filaggrin, are considered one of the strongest risk factors for the development of the disease [31,32]. It is important to note that not all patients with AD have filaggrin mutations and that some patients with them do not necessarily develop the disease, highlighting the fact that the development of AD is multifactorial resulting from a complex interaction between genetics and environmental conditions. As primary skin barrier defect may not be present in all patients with AD, it
is important to point out that AD may actually represent a clinical syndrome that could result from different mechanisms.

Recent research in dogs has highlighted that skin barrier impairment may be one of the mechanisms of allergic sensitization also in this species [33,34] when allergens come in contact with a more permeable and disrupted skin which results in a Th2 polarization and production of allergen specific IgE. Although it is not clear yet whether the skin barrier defect is primary in dogs, it is clear that the skin barrier function is impaired [35,36] in atopic dogs and it plays a role in the aggravation of the disease.

Interestingly, in people, AD is typically the first step in a progression of events called “atopic march” which starts in the skin and progresses in many, but not all cases, into respiratory signs (rhinitis and later on asthma) [37]. As mentioned above, it is important to point out that although this progression is noted in individual patients, debate exists on the evidence of this phenomenon. Some publications have questioned the existence of an actual atopic march [38,39] while more recent studies have provided renewed support of an actual temporal pattern of progression from eczema to allergic rhinitis and asthma and a correlation between early eczema and childhood asthma [40–42].

In dogs, atopic disease typically only manifests in the skin, without progression to respiratory signs in the vast majority of cases. On the contrary, in other domestic animals, such as cats and horses, the atopic disease can manifest as both dermatitis and respiratory disease. It is currently unclear why allergies have different forms of manifestations in various species and why some species are prone to skin manifestations while others are prone to respiratory disease. Allergic diseases have very complex pathogenesis and many genes have been considered as possible candidates to explain development of allergies. It is very likely that many different pathways can lead to the development of allergies and that even within one species allergies should be considered more like a syndrome rather than one disease. It is interesting to note that even within the canine species, different gene mutations may play a role in various breeds and various geographical areas [43]. As the recent literature has emphasized the role of increased penetration of allergens as one of the mechanisms to explain increased risk for sensitization, it is possible that different aberrations in the skin versus the respiratory epithelium may be one of the reasons for the various clinical manifestations of allergic disease in different species. The similarities and yet differences across species may actually constitute a key to understanding what determines the progression of the disease and hopefully allow early identification of the individuals that are at increased risk for the development of asthma.

Histologically and immunologically the similarity of AD between dogs and people is striking, making dogs the closest naturally occurring animal model to study this disease in people [44]. Atopy patch test reactions in dogs mimic naturally occurring lesions and are very similar to what is reported in human medicine [45]. AD type lesions may be experimentally induced in mice, but this species does not spontaneously develop the disease and the experimental models only reproduce limited aspects of the disease [46] and recent literature has questioned the value of mouse models [47] as mice appear to only reproduce a very simplistic approach to allergies as a Th2 mediated disease and fail to reproduce more complex aspects of the human condition [48]. Dogs, on the other hand, are genetically closer to people, they are spontaneously affected by AD, and they also share the same environment with their owners constituting a precious and better tool to fully understand this complex disease in people.
2.1. Dog Models of Atopic Dermatitis

A few canine models of AD have been identified and validated. One model is composed by a colony of high IgE producing atopic Beagles [49]. These dogs have skin barrier defects demonstrated by ultrastructural studies showing an abnormally organized lipid lamellae and retained lamellar bodies [50]. These changes are similar to what reported in human atopic patients in which disorganized intercellular lipids, decreased ceramides and defects of extrusion of lamellar bodies have been described [51,52]. The abnormalities in the skin barrier do not only affect lipids but also epidermal proteins. Filaggrin has also been studied in dogs, in particular in the atopic beagle colony mentioned above, and an irregular, patchy staining for filaggrin was found in immunohistochemical and immunofluorescence studies [53,54]. In addition, in histochemical studies, morphological differences have been identified when comparing the keratohyalin granules (granules where the filaggrin and its precursor are stored before complete keratinization) in the atopic beagles with age matched healthy controls. In people, the decreased expression of filaggrin is not always linked to genetic mutations, but it can also be caused by increased degradation of the protein. More specifically, two enzymes—caspase-14 and calpain-1—are responsible for the degradation of filaggrin into amino acids fundamental for proper miniaturization of the skin (natural moisturizing factors) [55]. A decreased expression of caspase-14 has been found in both people [56,57] and in the atopic Beagle [58] colony showing the relevance and the similarities between the canine model and the atopic condition in human patients.

In this Beagle model, the skin barrier defects are not only ultrastructural but also functional as demonstrated by an increased transepidermal water loss (TEWL) compared to age matched healthy controls [36]. Measurement of this parameter is a non-invasive approach to test skin permeability. Atopic people have increased TEWL when compared to healthy controls and skin barrier dysfunction correlates with disease activity [59]. It is believed that younger skin is more susceptible to sensitizations and interestingly puppies from this atopic colony have higher TEWL than adults of the same colony and age matched healthy Beagles kept in the same living conditions [36]. These findings seem to emphasize the existence of a window of opportunity of allergic sensitization. This leads to the fascinating hypothesis that maybe the development of AD could be prevented or at least minimized if skin barrier repair is enacted at a young age, before sensitization sets in. Indeed, pilot studies in infants showed a decrease sensitization in children at high risk for the atopic disease due to genetic predisposition in which emollients and occlusive therapy was started in the first few months of life [60,61]. Preventative studies are difficult to accomplish in people or in pet dogs with naturally occurring disease due to the variable environmental conditions which tend to make design and interpretation of studies quite challenging. That is where animal models can provide useful information that can benefit multiple species. One example of that is the proactive use of probiotics with the intention of preventing or at least lessening the severity of disease in highly predisposed individuals. One of the ideas behind this strategy is that allergies are partly due to improved hygiene conditions leading to less opportunity to have bacterial and parasitic exposure which helps to modulate the immune response toward a more protective cell mediated response. The exact mechanisms of probiotics in allergies are still under investigation and are more complex than the direct modulation of the immune response. With the development of methodologies targeting the 16S rRNA, knowledge of the importance of the microbiome in healthy and disease states is rapidly growing. It is becoming evident that loss of
biodiversity can predispose to the development of allergies while high biodiversity can have a protective effect [62–66]. Oral assumption of probiotics can modulate gut microbiota [67] and this can be an additional mechanism by which probiotic can affect the immune response [68], although this mechanism has not be confirmed in recent clinical trials in infants. In one study, skin and gut microbiome was investigated after oral administration of Lactobacillus rhamnosus GG (LGG) but no significant changes were detected when compared to the control group [69]. When studies on probiotic have been done in people, the results of clinical efficacy have been mixed [70] probably because it was not possible to control for genetic differences, variable diets and environmental conditions. In animal models, this can be done and even studies with a relatively low number of subjects can produce useful information. Using the colony of atopic Beagles, it was possible to breed the same parents twice, producing two litters, one used as control and the other one as probiotic litter. Puppies were raised under the same housing and dietary conditions, they were all exposed to house dust mites using the exact same doses and protocols and the only difference was the administration of LGG, which is a probiotic for both dogs and people. The results showed that prenatal and postnatal (for the first 6 months of life) exposure to LGG had a protective effect in decreasing the severity of sensitization [71]. Even more interestingly, the protective effect was long lasting and carried out for at least 3 years after the discontinuation of the LGG [72]. The probiotic litter continued to show significantly lower severity of clinical signs and was associated with increased regulatory cytokines such as transforming growth factor (TGF)-β. In the canine study, no investigation on the effects of LGG on skin or gut microbiome was done. This is an area that should be investigated further as preliminary studies on skin microbiome in allergic and normal dogs have shown that atopic dogs have less biodiversity and increased colonization by Staphylococcus compared to the healthy controls [73]. The preventative effects of this study echoed more recent metanalyses in human medicine that support a prophylactic role for probiotics, but not the therapeutic role once the disease has already been established [74].

A model for AD in dogs has also been described in Maltese-Beagles. In this model, pruritus and dermatitis can be elicited after epicutaneous application of house dust mites [75]. Lesions are consistent with naturally occurring AD lesions [76,77] making it a suitable model to test new therapies. No skin barrier defect has been described in this model compared to a control population. Ceramide reductions have been described as an effect of allergen exposure and development of inflammation [78]. Another well described but older model of AD and asthma was the one of Basenji-Greyhound dogs. While dogs typically do not develop asthma as progression of their skin disease, in this model both manifestations (skin and respiratory) of atopic disease were described [79] making them an appealing model to study the human condition [80,81]. No recent publications have been reported on this model.

2.2. Mouse Models of Atopic Dermatitis

Many different mouse models of AD have been described over time. The Nishiki-nezumi Cinnamon/Nagoya (NC/Nga) was the first AD-like murine model that spontaneously develops AD-like dermatitis only if raised under air-uncontrolled conventional conditions [82–84]. This model has been useful to test new treatments [85] although the fact that the NC/Nga mice exposure to only conventional environments is not enough to induce human AD-like clinical symptoms questions the relevance of such model to the human condition.
Other mouse models have been described by epicutaneous stimulation with certain environmental allergens [86]. SKH-1 hairless mice have been shown to be easily sensitized epicutaneously with allergens due to skin barrier impairment and reported to be helpful to test strategies such as probiotics [87]. Mice with a null mutation in the filaggrin gene develop flaky tails and that has been used as model for AD although this model may only represent one subset of AD [88,89].

3. Food Allergy

Food allergy is also becoming quite common in people. In people, food allergy is defined as “a pattern of immune reactivity to the ingestion of natural food components resulting in IgE mediated [reaction], which by nature routinely results in tolerance”. In non-allergic individuals, the normal immune response to dietary protein is associated with a natural tolerance; on the contrary, in allergic patients, an excessive complex immune response against food proteins may occur. Several immune responses including an immediate and a delayed hypersensitivity have been associated with food allergy explaining the highly variable time of reaction after the ingestion of an allergen. Food allergy is extremely important in people due to the high variability of the clinical signs associated with it; the spectrum of clinical signs can range from mildly irritating to life-threatening conditions [90].

Food allergy is a substantial and evolving public health issue [91,92] with an increasing prevalence in the last decades [93] due to environmental changes [94]. In the USA, up to 8% of children and 4% of adults have food allergy [92]. Of major concern is the fact that, while previous generations of children would typically outgrow food allergies (particularly if not peanuts related), the new generations appear less able to outgrow food allergy. It is proposed that environmental factors have significant effect on this phenomenon and that “westernization” plays a role [95]. The most serious allergic response to food allergy is anaphylaxis [96,97]. More than 3 million Americans are allergic to peanuts and this is the leading cause of death by anaphylaxis [98]. In children, the most common foods able to trigger an immunological response include, but are not limited to, cow’s milk, hen’s egg, peanut, soybeans, wheat, fish, and tree nuts. Whereas, in adults, the most common food allergies are due to ingestion of peanuts, tree nuts, fish, and shellfish [99–101]. In people, most commonly food allergy started in the first 3 years of life; however, several studies have shown that most allergies that begin early in life (e.g., milk, egg, soy, and wheat) are generally outgrown. On the contrary, allergies to peanut, tree nuts, fish and shellfish tend to persist for life [102,103].

To better determine the immunological mechanisms and in order to develop safe therapeutic options for children and adults affected by food allergy, the use of animal models is fundamental. To date, animal models have been identified with some success in small animals (mice, rats, and guinea pigs) as well as in large animals (dogs, pigs, and sheep). Those species have been fundamental to assess routes of administration to induce sensitization and to better identify the gastrointestinal allergic response. However, each model has benefits and limitations.

3.1. Rodent Models of Food Allergy

Examples of murine model include strains like the BALB/c, DBA/2, C3H/HeJ, BDF-1, A/J, and the C57/Bl6. Of those, the most studied have been the C3H/HeJ and the BALB/c able to produce IgE and IgG1 anaphylactic antibodies [104,105]. The former has been used to investigate potential
immunotherapeutic approaches to treat food allergy through intragastric administration of peanut and milk proteins [106,107]. The BALB/c mice have instead been used to evaluate the potential to predict the allergenicity of a novel protein for people through intraperitoneal administration of several proteins including ovalbumin, peanut agglutinin, and bovine serum albumin [108]. Due to the artificial sensitization methods it is unclear how well representative mouse models are for the natural disease in people and their usefulness in predicting efficacy of therapies [109].

Rats have also been used as an animal model for food allergy. The most commonly used strain is the brown Norway able to produce allergen specific IgE after oral sensitization and able to predict the allergenicity of novel proteins [110,111]. Other strains like Wistar, Hooded Lister, and Piebald Virol Glaxo have been studied, but not commonly used due to the inability to detect quantifiable levels of antigen specific IgE [112].

An alternative to mice and rats is the guinea pig. Indeed, these rodents are able to display fatal anaphylaxis after sensitization to cow’s milk proteins. However, due to the technical difficulties (e.g., inability to directly quantify IgE production) and for the limited tools to study their immune system, guinea pigs have been abandoned as an animal model for food allergy [113].

3.2. Dog Models of Food Allergy

Dogs have been recently identified as a spontaneous model for food allergy in people. In fact, as in people, up to 8%–15% of the canine population is spontaneously affected by food allergy and it manifests more commonly during puppyhood [114]. Like in people, dogs may present with skin and gastrointestinal clinical signs, although these latter involves up to 10%–15% of the affected patients [113,115]. Contrary to the rodent models, dogs and people share the same food allergens: beef, dairy, wheat, lamb, egg, chicken, soy, oats, and pork [116]. In addition, they share similar gut anatomy and physiology, as well as nutritional requirements. On dogs, it is easier performing repeated endoscopic analysis, high IgE dogs are easy to identify, and the large size of immune organs along with larger blood volume facilitate certain analyses [104]. To date, three canine models have been well validated [117–119]. All the models include mixed breed atopic dogs. Two models are constituted by the same colony of inbred, high IgE producing, atopic spaniel/basenji dogs [117,118], whereas the other one is constituted by a colony of Maltese/Beagles atopic dogs [104,120]. The first generation of the spaniel/basenji model was developed by subcutaneous injection of wheat, cow’s milk and beef extract showing signs of vomiting, diarrhea, or more rarely constipation [118]. The second generation was instead sensitized by subcutaneous injection of peanut, walnut, Brazil nut, wealth or barley developing vomiting and lethargy after oral challenge [117]. On the contrary, the Maltese/Beagle colony spontaneously developed food allergy (pruritus, diarrhea, and/or vomiting) to cow’s milk, dairy products, corn, and soy without any experimental sensitization [104]. In all three models, the investigators were able to show clinical response to oral challenge between 1 h and 12 h, a higher production of total and allergen-specific serum IgE, and positive intradermal test [104,117,118]. Using the canine model for food allergy, the investigators proposed the hypothesis that in genetically predisposed children an early infection may stimulate the immune system to respond to “bystander” antigens more aggressively than healthy individuals. In the affected patients, the inflamed gut becomes
more permeable to food allergens (proteins) leading to their exposure to the local immune system inducing sensitization of otherwise innocuous food proteins [105].

A recently described model for peanut allergy has been reported in atopic Beagles [121]. In this model, dogs were sensitized after epicutaneous application of a peanut paste and developed macular, popular dermatitis after oral challenge. No systemic effects were detected after oral challenge making it a safe model to test therapies. An IgE mediated response was detected on intradermal test and skin biopsies. This model could be useful to test new allergy vaccines to treat peanut allergy in children. The epicutaneous sensitization is very relevant to what is believed to occur in young atopic children where the perioral region is frequently one of the first affected areas, most likely due to increased penetration of food allergens through an impaired skin barrier [122].

### 3.3. Other Large Animal Models for Food Allergy

Another large animal model proposed for food allergy is constituted by neonatal pigs. This model, although very similar to the canine model, is much less commonly used due to several limitations. The major benefits of this model over the others include the strong similarity in anatomy and physiology of the gastrointestinal tract and the development of mucosal immunity between pigs and people [105]. Neonatal piglets have been used as an animal model for sensitization to cow’s milk, soy, and peanuts. Sensitized piglets show a very similar immunological response occurring in sensitized children [105]. The induction of food sensitization in piglets involves the use of intraperitoneal injections (peanut extract) in the presence of cholera toxin as adjuvant and later on repeated oral challenges with sensitizing food allergen [105,123]. After challenge the pigs show clinical signs including vomiting, lethargy, diarrhea, bleeding, weight loss, and respiratory distress [123]. Finally, like in dogs, the clinical signs are associated with an increase in allergen-specific IgE and IgG, and a positive passive cutaneous anaphylaxis showing the involvement of IgE as cause of the allergic symptoms [105]. The last large animal model proposed for food allergy in people involves the use of sheep. Like dogs and pigs, also sheep are very similar in size and physiology to people. Sheep have been successfully used as model for peanut allergy [111]. Similarly to other animal models, sheep were sensitized by multiple subcutaneous injections of crude peanut extract and ovalbumin. As outcome the investigators were able to show the presence of Ara h 1- and Ara h 2-specific serum IgE in 100% and 80%, respectively, of the sensitized sheep and a positive intradermal test in 80% of the high-IgE producing sheep [111].

### 4. Asthma

Asthma in people is defined as “a [multiphenotypic] chronic inflammatory disorder of the airways associated with airway hyper-responsiveness [leading] to recurrent episodes of wheezing, breathlessness, chest tightness, coughing, [and] airflow obstruction often reversible with or without treatment” [124–126]. Asthma affects more than 10% of the North American population with the age of onset, pathophysiology, and response to treatment extremely variable [127,128]. In addition, a correlation between chronic obstructive pulmonary disease (COPD) has also been proven in patients with asthma [129].
Due to the high heterogeneity and the ethical problems in analyzing the pathomechanism of asthma in children, many disease models have been developed (mathematical modelling system as well as in vitro and in vivo models) [130]. The mathematical model has been used to describe patterns of flow limitations in the airways [131], whereas understanding of cell signaling, cellular responses under Th2 conditions, and wound repair have been facilitated by the use of primary or immortalized cell lines [132–134]. Although these two model systems have been largely used and are fundamental to unravelling the physical and immunological processes at a cellular and molecular level, their major limitation is the impossibility to evaluate such processes in a dynamic immunological milieu present in the asthmatic patient [130]. For this reason, the only model system able to reproduce the asthma in people is the animal (in vivo) model. In the past 100 years, the use of animal models have largely contributed to the identification of important immunological alterations in the lungs of asthmatic patients (e.g., the importance of Th2 phenotype in the progression and perpetuation of an inflammatory response in allergic asthma) as well as the development, safety, and efficacy of new drugs (e.g., montelukast, integrin antibodies, and tryptase inhibitors) [130].

Contrarily to AD and food allergy, an appropriate animal model for human asthma is lacking. Indeed, to date, a laboratory animal that spontaneously develops a disease with characteristics present in asthma has not been identified [135]. However, different species have diseases with some similarities to the human disease; cats spontaneously develop a bronchial disease similar to chronic asthma, horses are affected by a neutrophilic-dominated airway disease more close to COPD than asthma, sheep and dogs are naturally susceptible to allergen sensitization [135].

Mouse models have also been developed and use for identification of immunological alterations leading to pulmonary inflammation in asthmatic people; however, this model is not ideal for testing of new drugs due to the significant anatomic and immunological differences between mice and people [135,136]. Like mice, rats have also been largely used as animal model due to the abundance of reagents available, the ease handling and sensitization protocols, as well as the low maintenance expense. However, one advantage over mice is their larger size which determines an increase in sampling volume and possibility of testing compared with mice [135]. In addition, rats are able to develop an early- and a late-phase response as well as airway hyperresponsiveness to non-specific bronchoconstricting agents present in people but not in mice [135,137]. Although extremely useful to investigate the acute allergic reaction in asthma, the murine models have been largely criticized for their ability to easily develop tolerance following allergen sensitization limiting the use of such models for the investigation of chronic allergic response in asthmatic patients [135].

Contrary to the murine models, pet cats are spontaneously affected by a syndrome similar to human asthma [138]. In the past 10 years, such similarities have led to the development of two feline asthma models for preclinical studies [139–141]. Like in people, in cats asthma is extremely common and triggered by aeroallergens and the clinical manifestations include cough, wheeze and episodic expiratory respiratory distress [138]. In addition, cats with asthma have important features of asthma in people including recurrent and variable symptoms of chronic airway disease, airflow restriction, bronchial hyperresponsiveness, and airway inflammation [142]. However, contrarily to people in which airway hyperresponsiveness is triggered by drugs, pollutants, occupational factors, exercise, emotional stress, infections, and allergens; in cats the predominant triggers seem to be aeroallergens [138].
Like the feline models, the canine model has been identified as a better model due to the natural predisposition of dogs to develop allergic disease after exposure to allergens clinically relevant for people. However, naturally-occurring asthma is very uncommon in allergic dogs [143]. In fact, the most common clinical manifestations of allergy in dogs include dermatitis, otitis, and conjunctivitis [144]. However, many naturally or actively sensitized dogs to *Ascasis suum* larvae or ragweed have been used as canine model for asthma [134]. Although an increase in eosinophils have been identified in the airways of allergic dogs, this does not seem to be associated with an increase in responsiveness of the airways probably due to the significant anatomical difference of dogs with the other species; proportionally larger airways and less prone to bronchoconstriction [145]. Nevertheless, anatomical and clinical differences are evident between dogs and people; such model has been largely used due to the unique ability of dogs to show persistent sensitization (prolonged airways hyperresponsiveness) up to 5 months post challenge with *A. suum* [146]. The main drawback for this model is that dogs are labor intense and expensive, but these difficulties are easily overridden by the usefulness of such model to identify pathomechanisms and long term pulmonary changes related to the asthmatic disease [134]. The canine model has also been used as animal model for COPD in people. Although, no as much research as in asthma has been involved using the canine model, three model systems have been identified in dogs: inhalation of cigarette smoke, exposure to SO$_2$ gas and intratracheal or aerosolization of proteolytic enzymes [125].

Finally, the last animal model system used for asthma is sheep. Like dogs, sheep are naturally sensitized to *A. suum* and house dust mite [135,147,148]; although an inter-individual variability to mount an allergic physiological response to the allergen is present among sheep [149]. Similar to dogs, after challenges sheep develop an early- and late-phase as well as airway hyperresponsiveness [135]. In term of drug response, cromolyn and corticosteroids are highly effective in sheep with an allergic airway response [147]. In addition, sheep are more similar in size and physiology to people, they are very docile, and fewer ethical constraints are taken [147,150]. For all the reasons abovementioned sheep represent a very useful model system for asthma in people; however, relevant differences between sheep and people may affect the use of such model for development and testing of new drugs. One example is the efficacy of platelet activating factors extremely useful to modulate the inflammatory response in the sheep model, but not at all effective in people [151]. As in dogs, the main difficulties to work with sheep are the expense and the labor intensity; however, like for the canine model, such difficulties are easily overridden by the advantages of such model. Some key advantages of using large animal models include their outbred nature, allowing studies that are more comparable to humans, the ability to conduct serial experiments within the same cohort of animals, and their relative longevity, allowing more relevant investigations into chronic disease as well as the long-term evaluation of specific therapies.

5. Conclusions

Animals and people share many important allergic diseases, and animals have proven to be the key to improve our understanding on the mechanisms involved across species for the mutual benefit of people and animals. Animals and people share the same environment and are inextricably linked. A comparative approach to medicine has improved our appreciation of similarities and differences of
diseases that affect multiple species and how that can be leveraged to improve our knowledge of the mechanisms involved. It is hoped that by improving our understanding on how we are all connected, veterinarians and physicians can collaborate across species to improve the lives of all species and develop therapies than can benefit multiple species.

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Author Contributions

Domenico Santoro and Rosanna Marsella equally contributed to the writing, revisions and editing of the paper. They are both veterinary dermatologists with extensive experience in animal models of allergy.

Conflicts of Interest

The authors declare no conflict of interest.

References


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