



Translational value of animal models of asthma: Challenges and promises

Seil Sagar*, Hamid Akbarshahi, Lena Uller

Unit of Respiratory Immunopharmacology, Department of Experimental Medical Science, Lund University, Sweden

ARTICLE INFO

Article history:

Received 5 December 2014

Received in revised form

22 January 2015

Accepted 12 March 2015

Keywords:

Ovalbumin

House dust mite

Severe asthma

Exacerbation

Viral infection

ABSTRACT

Asthma is a heterogeneous disease in which various environmental stimuli as well as different genes, cell types, cytokines and mediators are implicated. This chronic inflammatory disorder of the airways is estimated to affect as many as 300 million people worldwide. Animal models of asthma, despite their limitations, have contributed greatly to our understanding of disease pathology and the identification of key processes, cells and mediators in asthma. However, it is less likely to develop an animal model of asthma that takes into account all aspects of human disease. The focus in current asthma research is increasingly on severe asthma because this group of patients is not well treated today. Recent advances in studies of asthma exacerbation are thus considered. We therefore need to develop translational model systems for pharmacological evaluation and molecular target discovery of severe asthma and asthma exacerbations. In this review we attempted to discuss the different animal models of asthma, with special emphasis on ovalbumin and house dust mite models, their merits and their limitations.

© 2015 Elsevier B.V. All rights reserved.

1. Animal models of asthma

Asthma is predominantly a human disease. With possible exception of cats and horses, animals do not spontaneously develop asthma-like conditions (Holmes et al., 2011; Mullane and Williams, 2014; Zosky and Sly, 2007). A bronchial disease similar to human chronic asthma was reported to occur in 1% of cats (Padrid, 1992). Upon exposure to moldy hay horses can develop heaves as a naturally occurring neutrophil dominated obstructive pulmonary disease (Herszberg et al., 2006; Leclerc et al., 2011). Similar to human asthma, heaves is further characterised by chronic airway inflammation, airway hyperresponsiveness (AHR) and reversible bronchoconstriction (Leclerc et al., 2011). Horses with heaves were also demonstrated to have airway smooth muscle remodelling comparable to human asthmatic airway remodelling (Herszberg et al., 2006). However, ethical reasons and obvious practical and technical issues limit the use of horses and cats as animal models of asthma.

Guinea pigs are one of the earliest species used in studies of allergic respiratory disease (Karol, 1994). The development of a well-characterised early phase and late phase airway responses upon allergen sensitisation and challenge makes these animals a suitable model for asthma (Ressmeyer et al., 2006; Zosky and Sly, 2007). Similar to human severe asthma, guinea pigs also develop profound

breathing difficulties associated with pulmonary inflammation composed of both eosinophils and neutrophils (Shin et al., 2009). Interestingly, eotaxin, an eosinophil chemoattractant, was in fact first discovered in guinea-pigs (Jose et al., 1994). However, the shortage of inbred strains and the low number of species-specific reagents available limit the use of guinea pigs in asthma research (Ricciardolo et al., 2008).

Rats, rabbits, dogs, monkeys and sheep have also been utilised in models of allergic airways disease (Shin et al., 2009; Zosky and Sly, 2007). Each of these animals has its own advantages and disadvantages as a model of allergic asthma (Shin et al., 2009).

The availability of transgenic animals and a large selection of specific reagents and techniques have made the mouse the 'gold standard' species for asthma research (Daubeuf and Frossard, 2014). There may be particular translational power in research approaches that combine mouse model studies with mechanistic studies involving human primary bronchial cells from asthmatic patients (Uller et al., 2010). BALB/c mouse is the most commonly used strain for allergen challenge models as these mice are more prone to develop a T helper type 2 (Th2) immune response upon allergic sensitisation and challenge. Also used in allergen challenge models are the C57BL/6 and A/J strains (Kumar et al., 2008).

Here we discuss briefly current trends in asthma research of relevance to the sections that follow dealing with experimental mouse models of asthma. The focus in asthma research is increasingly on severe asthma because this is where medical need is unmet. Recent advances in studies of asthma exacerbation are

* Correspondence to: Department of Experimental Medical Science, BMC D12, Lund University, 221 84 Lund, Sweden. Tel.: +46 462227763.

E-mail address: seil.sagar@med.lu.se (S. Sagar).

thus considered. Some of the demands we may have on models of severe asthma are underscored because they concern aspects of the disease that are not well treated today.

2. Asthma

Asthma is a chronic inflammatory disorder of the airways with increasing incidence and prevalence throughout the world, especially in children and within developing countries (Weinberg, 2011). According to the Global Burden of Asthma report, an estimated 300 million people have asthma worldwide (van Schayck, 2013). The disease is characterised by chronic pulmonary inflammation, episodes of reversible airway narrowing and AHR (McMillan and Lloyd, 2004). Structural changes in the airway walls of asthmatic subjects, referred to as “airway remodelling”, may develop within days after allergen exposure but are generally thought of as a consequence of persistent chronic inflammation and exaggerated repair of injured tissue (Persson, 2014; Shifren et al., 2012). Whilst the majority of asthmatic subjects are well controlled with inhaled corticosteroids and long-acting beta agonists, about 10% of the asthmatic population are poorly controlled with these mainstay drugs. Particularly worrisome is the 5–10% of asthmatics who suffer from severe asthma. These patients also account for a dominating part of the health care costs for asthma (Chung et al., 2014).

It is now well accepted that asthma is a complex multifactorial disorder with different phenotypes underlined by different pathological mechanisms (Expert Panel Report 3, 2008 Holgate, 2012; Yawn, 2008). Further discoveries of important mechanisms of asthma will hopefully make it possible to define specific asthma endotypes amenable for specific and selective therapeutic interventions (Chung, 2014; Chung et al., 2014). Unravelling of detailed molecular and cellular features of distinct asthma endotypes would be immensely important for advancing the experimental research that involves animal models as well as in vitro studies using human diseased cells. However, even regarding basic and well-established pathological features of severe asthma and asthma exacerbations, such as occurrence of eosinophil degranulation, epithelial derangement and sub-epithelial microvascular participation, improvement of current models of asthma is warranted (Persson, 2002).

The limitations of the current treatments for asthma highlight the unmet need for effective therapies with improved clinical efficacy to accomplish disease control. In this regard, the development of clinically relevant animal models is warranted.

3. OVA asthma models

Animal models have become an invaluable tool in translational research. In particular, mouse models have made major contributions to our understanding of asthma pathophysiology (i.e. contribution of different cytokines, chemokines and receptors) and the modelling of specific features of this disease (Lloyd, 2007; Nials and Uddin, 2008).

To date, the protein ovalbumin (OVA), derived from egg white, is still the allergen of choice in asthma models (Mullane and Williams, 2014). Exposure of the animals to OVA via the airways produces an airway inflammation model that exhibits a number of human asthma-like cellular and pathophysiological features (i.e. increased AHR, cellular inflammation and increased levels of inflammatory cytokines in bronchoalveolar lavage (BAL) fluid) (Nials and Uddin, 2008). In this regard, it should be realized that increased cellular inflammation is not always correlated to increased AHR in both severe asthmatics and animal models (Manni et al., 2014).

However, the concern about the applicability of the OVA mouse models continues to grow. Prolonged exposure to OVA can induce tolerance in the animals resulting in reduced airway inflammation

and AHR (Jungsuwadee et al., 2004; Kumar et al., 2008). The induction of tolerance can be influenced by factors like the strain of mouse used and the route of allergen administration (McMillan and Lloyd, 2004; Shinagawa and Kojima, 2003).

Since the antigen OVA is not associated with asthma in humans, antigens like house dust mite (HDM), grass pollen and cockroach extracts with a greater clinical relevance have also been employed (Fuchs and Braun, 2008; Johnson et al., 2004; Kim et al., 2006; Sarpong et al., 2003). House dust mite models of asthma will be discussed in Section 4. Concurrent dual allergen exposure models of asthma have also been developed to resemble the continuous exposure of human asthmatics to a variety of environmental stimuli. Mice exposed to a combination of OVA and HDM did not show more robust allergic airway disease than mice exposed to either allergen alone (DiGiovanni et al., 2009; Fattouh et al., 2005).

The “classical” OVA challenge model, which has yielded rather consistent responses in the many different sensitisation and challenge protocols, involves peripheral sensitisation followed by challenge with OVA in the presence or absence of an adjuvant (Nials and Uddin, 2008). For the sensitisation, peritoneal, subcutaneous or dermal routes are used. Allergen challenge is carried out via the airways (aerosol or intratracheally) or intranasally (Lloyd, 2007; Mullane and Williams, 2014). Adjuvants such as the most commonly used aluminium hydroxide potentiate the immune system for the development of an antigen-specific Th2 immune response (Exley et al., 2010). Endotoxins, ozone, exogenous proteases, diesel exhaust and cigarette smoke have also been utilised as adjuvants in mouse models of asthma (Wegmann and Hauber, 2010). In addition, adjuvant-free protocols have also been described (Conrad et al., 2009).

A comprehensive overview of mouse models of acute and chronic allergic pulmonary inflammation is given in Martin et al. (2014) and Nials and Uddin (2008).

Despite the concerns about the utility of OVA models in asthma research, these models have contributed to our understanding of the key role played by T cells and Th2-associated cytokines interleukin (IL)-4, IL-5 and IL-13 in disease initiation and progression (Brusselle et al., 1995; Cohn et al., 1997; Fan et al., 1997; Hamelmann et al., 1999; Kumar et al., 2002; Leong and Huston, 2001; Muller et al., 1993; Shardonofsky et al., 1999; Walker et al., 1998; Webb et al., 2003; Wegmann, 2008; Wegmann and Hauber, 2010). Accordingly, one of the translational values of OVA asthma models is a range of therapeutics targeting the Th2 inflammatory response in the disease including receptor-homologous molecule expressed on Th2 cells (CRTH2) antagonists (Uller et al., 2007) and antibodies to IL-5 and IL-13 which have had some promising effects in the clinic (Berair and Pavord, 2013; Corren et al., 2011; Mullane, 2011). Most importantly, monoclonal anti-IL5 antibody treatments have been demonstrated to have clear therapeutic roles in subgroups of severe asthma exhibiting eosinophilia including those patients with severe asthma combined with nasal polyposis (Berair and Pavord, 2013).

4. HDM asthma models

Different human allergens have been used recently in mice and other species to make the allergens more clinically relevant. Extracts or purified proteins from allergens such as fungi (e.g. *Aspergillus fumigatus*), HDM, cockroach, ragweed and pollen spores, are being used. (Barrett et al., 2003; Chapoval et al., 2002; Fuchs and Braun, 2008; Lambert et al., 1998; Warner et al., 2004).

HDMs (*Dermatophagoides* sp.) are one of the most common allergens worldwide. 50–85% of asthmatics are typically HDM allergic and have elevated levels of HDM-specific IgE. Early and late asthmatic responses have been observed after HDM challenge in these asthmatic subjects (Hatzivlassiou et al., 2010; Maunsell et al., 1968; Nelson et al.,

1996; Roche et al., 1997). In addition to asthma, other common allergic disorders caused by HDM are rhinitis, rhinoconjunctivitis and atopic dermatitis (Gavino et al., 2008; Platt-Mills et al., 1995; Stevenson and Birrell, 2011; Thomas et al., 2002; Valerio et al., 2005).

Dermatophagoides pteronyssinus and *Dermatophagoides farinae* can produce allergenic proteins. They also contain bacterial and fungal products such as lipopolysaccharide (LPS) and β -glucan. This may be due to microbial contamination or endosymbiotic bacteria (De Luna et al., 2009; Douwes et al., 2000; Gregory and Lloyd, 2011; Jacquet, 2011; Valerio et al., 2005).

Proteolytic activity of HDM and involvement of toll-like receptor (TLR) 4 signalling pathway in response to HDM exposure have been shown to be essential in HDM-induced inflammation (Asokanathan et al., 2002; Hammad et al., 2009; King et al., 1998; Phipps et al., 2009; Stevenson and Birrell, 2011; Sun et al., 2001; Trompette et al., 2009). While allergen-specific CD4⁺ Th2 cells have been considered as the main players in HDM allergic pathogenesis, recent studies have shown the role of the innate immune system to be crucial, as well (Gregory et al., 2009; Gregory and Lloyd, 2011; Johnson et al., 2004; Stevenson and Birrell, 2011).

Usually, HDM is administered topically into the airways, intranasally or intratracheally, over weeks. Repeated administration of this allergen leads to airway inflammation and eosinophilia. However, whether this inflammation is an allergic inflammatory response or a result of repeated nasal exposure to an inflammatory concoction is not clear. Repetitive exposure to HDM makes it impossible to differentiate between “sensitisation” and “challenge” (Birrell et al., 2010).

To overcome the limitation of OVA models of tolerogenic response during prolonged administration, the use of HDM has allowed us to investigate changes associated with chronic, persistent allergen exposure. Prolonged exposure to HDM led to remodelling of the airways with increased mucous cell density and AHR, which remained elevated for a while after discontinuation of HDM exposure (Johnson et al., 2004). Despite showing some remodelling features, there are some shortcomings of the HDM mouse model of asthma. Having fewer mast cells in the epithelium or the airway walls compared to humans is one example (Shin et al., 2009).

Another feature of HDM models, in contrast to both OVA models and human asthma, is the serum levels of specific IgE. IgE response in HDM models is weak while in asthmatics allergic to HDM the levels are much higher compared to non-allergic controls (Birrell et al., 2010; Johnson et al., 2004).

Mouse models of asthma suffer from lack of complete translatability due to anatomic and physiologic differences between mice and humans. In addition to mice, house dust mite allergens have also been used in sheep and non-human primates (Bischof et al., 2003, 2008; Koumoundouros et al., 2006; Snibson et al., 2005). Sensitised rhesus monkeys (*Macaca mulatta*) exhibited respiratory signs like cough and rapid shallow breathing along with increased airway resistance, during aerosol challenge with HDM. They had increased levels of HDM-specific IgE in serum, numbers of eosinophils and exfoliated cells within lavage. In addition, these monkeys also showed mucus cell hyperplasia, interstitial infiltrates of eosinophils, and thickening of the basement membrane zone (Schelegle et al., 2001).

HDM models can be used as an acute model to study cell and cytokine profiles which are usually modified in early stages, or as an extended model to study remodelling features in later stages. Chronic HDM models translate some aspects of human asthma including increased eosinophils and neutrophils and more mixed T-cell phenotype (Mercer et al., 2015).

5. Modelling severe asthma and disease exacerbations

Severe asthma largely equals the significant asthma that is not well treated today (Chung et al., 2014). Hence, there is a pronounced

medical need for mouse models of severe asthma, including severe exacerbations of the disease. As shown by the example of anti-IL5 drugs the common mouse model approach may contribute to development of drugs for subgroups of severe asthma. However, novel mouse models mimicking important features of severe asthma, and exacerbations of asthma, are clearly warranted. Current mouse models of allergic airway inflammation do not reflect well the situation in severe asthma with occurrence of dramatic degranulation of eosinophils, epithelial injury and neutrophilia in various combinations. Only with the severe epithelial and granulocytic pathology in place we may be able to learn about causative immunopathogenic pathways. Thus, we have limited information from mouse models regarding mechanisms involved in the more serious pathology of asthma. Development and severity of asthma has been demonstrated to be associated with viral and bacterial respiratory infections in both early and later life (Guilbert and Denlinger, 2010; Hansbro et al., 2012). In this regard, mouse models are currently being developed addressing the role of infection in severe asthma and disease exacerbations (Starkey et al., 2013).

Acute asthma exacerbations, due to viral-induced inflammation, are a major cause of morbidity, mortality and health-care costs. The majority of viral-induced exacerbations are caused by rhinovirus infection. There is a major interest in developing animal models for viral-induced asthma exacerbation. One main issue in addressing this problem is that 90% of rhinovirus serotypes (major receptor group) do not bind to the mouse intercellular adhesion molecule-1 (ICAM-1) receptor, which has hampered the development of rhinovirus-induced mouse asthma models. Researchers are therefore restricted to using minor group viruses such as human rhinovirus serotype 1b (HRV1b) for their studies. Using the OVA model to establish allergic inflammation, and infection by HRV1b as an additional challenge, Bartlett et al. (2008) have developed an acute exacerbation model of asthma. They observed features mimicking viral-induced exacerbation in patients with asthma including increased eosinophils, neutrophils, AHR and Th2 cytokines in this model. Other groups used the chronic HDM model but they did not observe an exacerbated phenotype with HRV1b (Rochlitzer et al., 2014). These findings emphasise the necessity of developing reproducible asthma exacerbation models that provide opportunities for pharmacological intervention studies.

Technical challenges in developing a reproducible rhinovirus infection in mice, together with the constraint of using the minor group or humanised ICAM-1 mice limit the successful use of rhinovirus infections. Influenza and respiratory syncytial virus (RSV) may also be used for challenge in mouse models but the relative role of these infections is debated (Mori et al., 2013). One significant approach circumventing problems and reproducibility with live rhinovirus is to use polyinosinic-polycytidylic acid (poly I:C) which is as a mimic of the double-stranded (dsRNA) molecules being formed during rhinoviral replication. Poly I:C has been used extensively as a rhinoviral mimic in experiments with human lung cells in vitro (Calven et al., 2013; Uller et al., 2010) and recently also as a challenge agent in human airways (Brandelius et al., 2014). Poly I:C may well represent a challenge agent with translational features as an exacerbation agent. For example, overexpression of the Th2 type cytokine thymic stromal lymphopoietin was induced by poly I:C in both asthmatic bronchial epithelial cells and in mice with established OVA-induced allergic airway inflammation (Mahmutovic-Persson et al., 2014). In the study by Mahmutovic-Persson et al. (2014) several additional signs of aggravated inflammation were induced by poly I:C challenges such as the neutrophilic chemokine CXCL1/KC and cytokine tumour necrosis factor alpha (TNF-alpha), increased BAL fluid and lung tissue neutrophilia. Importantly, the necrosis marker lactate dehydrogenase (LDH), reported to be a clinical marker of asthma exacerbations (Wark et al., 2002), was found to be induced only in mice with an exacerbation.

5.1. Future models of severe asthma

Numbers of eosinophils in bronchi or blood are a mainstay measurement in asthma and in mouse models of asthma. However, there may be large groups of asthmatics, including poorly controlled asthma, where numbers of eosinophils do not associate with disease features but there is a remarkable association between severity of disease and occurrence of free eosinophil granules and their released proteins (Persson and Uller, 2014). This clinically based observations open novel avenues of research on anti-asthma targets, mouse models of asthma, and studies of human eosinophils. Thus, we have suggested that novel mouse models of asthma are needed where eosinophil activity is reflected by higher numbers of cells in the lung (Persson and Uller, 2014). An early model of asthma exacerbation involved treatment of mice with established allergic inflammation with anti-FAS (a member of the TNF-alpha superfamily) mAb to induce apoptosis of inflammatory cells (Uller et al., 2005). However, instead of promoting inflammation resolution severe signs of aggravated inflammation were observed in mice receiving anti-FAS mAb treatment. These animals developed severe bronchial eosinophilic, epithelial, and neutrophilic inflammation demonstrating also that it should be possible to develop mouse models where both eosinophilia and eosinophil degranulation occur in mouse lungs (Uller et al., 2005). Importantly, the mode of degranulation was primary lysis of eosinophils (Persson and Uller, 2014; Uller et al., 2005). This mechanism involves chromatolysis and cell membrane rupture causing the release of protein-rich eosinophil granules in the lung tissue. Furthermore, this is the dominating mode of eosinophil degranulation as occurs in severe asthma (Persson and Uller, 2014).

Current mouse models of asthma not only lack cytolytic eosinophils. Asthma-like epithelial derangement is also lacking. This is not surprising since lysis of eosinophils, with ensuing release of toxic proteins, may cause epithelial derangement in asthma (Persson and Uller, 2014). Again, in the anti-FAS exacerbation model both eosinophil lysis and epithelial derangement are pronounced (Uller et al., 2005). Lysis of eosinophils and epithelial injury, shedding and repair are hallmark aspects of asthma and, hence, warranted components of future mouse models of severe asthma.

6. Merits and limitations of mouse models of asthma

Depending on the questions being asked, mouse models do replicate a number of features of human allergic airway disease making these models a good tool to study human asthma in an in vivo system (Takeda and Gelfand, 2009). That said, there are many important differences in anatomy, physiology and pathology of the airways and lungs between humans and mice. Size of the lungs, airway branching pattern, division of the lungs, the less prominent role of mast cells and immunoglobulin E in mouse models compared to the situation in humans and over-representation of eosinophils in mouse BAL fluid (40–80%) compared to asthmatic subjects (1–5%) are only a few examples of these differences (Epstein, 2004; Kumar and Foster, 2012; Mullane and Williams, 2014; Wegmann and Hauber, 2010; Wenzel and Holgate, 2006). In addition, one of the most important limitations of mouse models of asthma is that mice do not spontaneously develop asthma, but asthma-like features can be induced by exposure of the animals to an allergen (Bates et al., 2009; Uller et al., 2007).

Acute allergen challenge models have been extensively employed in asthma research to investigate hallmark features of the disease like pulmonary inflammation and AHR (Nials and Uddin, 2008). However, most models described so far are more suitable for investigating acute inflammation and they do not represent the chronic characteristics of the disease. Chronic allergen challenge models were shown to reproduce some of the structural changes (airway remodelling features)

seen in the airways of chronic asthmatics with modest degree of AHR (Bates et al., 2009; McMillan and Lloyd, 2004; Wegmann, 2008). Nevertheless, these models showed limited success either due to the development of tolerance in OVA models or due to usage of low doses of allergen to avoid tolerance resulting in mild response (Mullane and Williams, 2014). In addition, development of good mouse models of chronic asthma poses difficulties due to the absence of environmental cofactors such as pollution, lung infection and allergens (Epstein, 2006).

A limitation of mouse models of asthma is the fact that most of, if not all, the animal models used to study this disease are based on a Th2-driven disease phenotype while 50% of asthmatics suffer from atopic airway disorder without a Th2-mediated immune response or with quite mixed responses (Martin et al., 2014). Another consideration is that humans can have AHR even in absence of symptoms while the induced AHR in mice is transient (Bates et al., 2009; Daubeuf and Frossard, 2014).

Perhaps due to the short life span of small animals truly relevant chronic asthma models cannot be developed into perfection. Currently there is instead helpful focus on a variety of factors, including viral

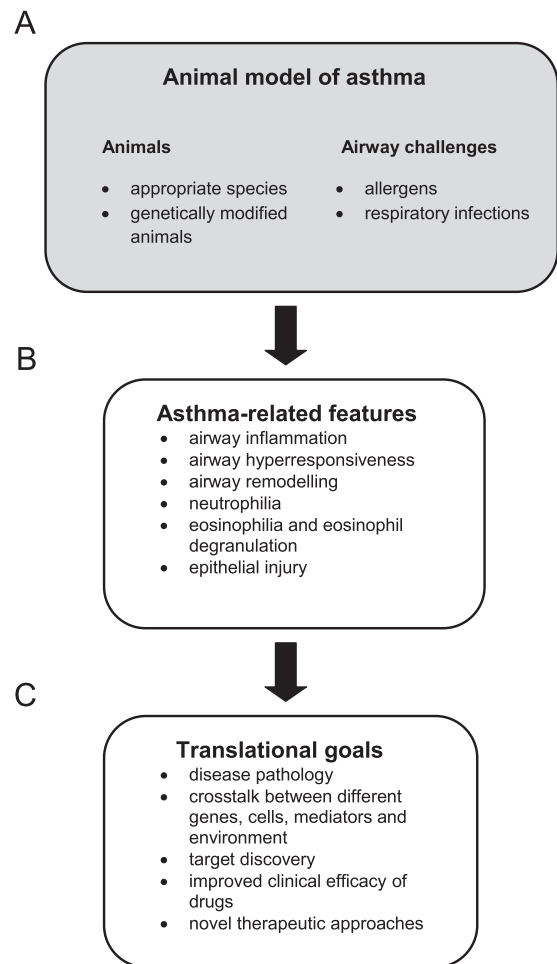


Fig. 1. Proposed translational animal model of asthma. (A) to develop an animal model of asthma with a translational potential, appropriate species, clinically relevant allergens or respiratory infections are needed. Use of genetically modified mice makes it possible to examine the role of a single gene, cell type or mediator in disease pathology. (B) The model should exhibit the important asthma-related cellular and pathophysiological features as well as severe asthma and disease exacerbation characteristics such as eosinophilia and eosinophil degranulation, and epithelial injury which are lacking in most, if not all, animal models of asthma. (C) The asthma-related features described in B should be translatable to the clinic by increasing our understanding of disease pathology, improving efficacy of new or existing drugs, the discovery of potential drug targets and the development of novel therapeutic approaches.

induced exacerbations that contribute to development of severe asthma.

7. Concluding remarks and future goals

Asthma is a complex multifactorial disease with different clinical phenotypes. It is unfair to demand that an animal model can mimic or translate all features of this heterogeneous disease as different genes, cell types, and mediators are implicated in asthma pathogenesis. Animal models of asthma have contributed significantly to our views of disease pathogenesis and have been instrumental in developing novel treatments such as anti-IL5 drugs. However, all animal models utilised in asthma research have their limits that have to be considered and realized before choosing a suitable model and when interpreting the outcomes of the study. It might be more appropriate and realistic to use different models to examine the role of specific pathways involved in the disease. Further development of mouse models of severe asthma is warranted, a proposal of a translational animal model of asthma is shown in Fig. 1. To improve translation power, the in vivo mouse model approaches can preferably be combined with human cells obtained from healthy and diseased donors.

References

- Asokanathan, N., Graham, P.T., Stewart, D.J., Bakker, A.J., Eidne, K.A., Thompson, P.J., Stewart, G.A., 2002. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J. Immunol.* 169, 4572–4578.
- Barrett, E.G., Rudolph, K., Bowen, L.E., Muggenburg, B.A., Bice, D.E., 2003. Effect of inhaled ultrafine carbon particles on the allergic airway response in ragweed-sensitized dogs. *Inhal. Toxicol.* 15, 151–165.
- Bartlett, N.W., Walton, R.P., Edwards, M.R., Aniscenko, J., Caramori, G., Zhu, J., Glanville, N., Choy, K.J., Jourdan, P., Burnet, J., Tuthill, T.J., Pedrick, M.S., Hurle, M. J., Plumpton, C., Sharp, N.A., Bussell, J.N., Swallow, D.M., Schwarze, J., Guy, B., Almond, J.W., Jeffery, P.K., Lloyd, C.M., Papi, A., Killington, R.A., Rowlands, D.J., Blair, E.D., Clarke, N.J., Johnston, S.L., 2008. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* 14, 199–204.
- Bates, J.H., Rincon, M., Irvin, C.G., 2009. Animal models of asthma. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L401–L410.
- Berair, R., Pavord, I.D., 2013. Rationale and clinical results of inhibiting interleukin-5 for the treatment of severe asthma. *Curr. Allergy Asthma Rep.* 13, 469–476.
- Birrell, M.A., Van Oosterhout, A.J., Belvisi, M.G., 2010. Do the current house dust mite-driven models really mimic allergic asthma? *Eur. Respir. J.* 36, 1220–1221.
- Bischof, R.J., Snibson, K., Shaw, R., Meeusen, E.N., 2003. Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. *Clin. Exp. Allergy: J. Br. Soc. Clin. Immunol.* 33, 367–375.
- Bischof, R.J., Snibson, K.J., Van Der Velden, J., Meeusen, E.N., 2008. Immune response to allergens in sheep sensitized to house dust mite. *J. Inflamm.* 5, 16.
- Brandelius, A., Andersson, M., Uller, L., 2014. Topical dsRNA challenges may induce overexpression of airway antiviral cytokines in symptomatic allergic disease. A pilot in vivo study in nasal airways. *Respir. Med.* 108, 1816–1819.
- Brusselle, G., Kips, J., Joos, G., Bluethmann, H., Pauwels, R., 1995. Allergen-induced airway inflammation and bronchial responsiveness in wild-type and interleukin-4-deficient mice. *Am. J. Respir. Cell Mol. Biol.* 12, 254–259.
- Calven, J., Yudina, Y., Uller, L., 2013. Rhinovirus and dsRNA induce RIG-I-like receptors and expression of interferon beta and lambda1 in human bronchial smooth muscle cells. *PLoS One* 8, e62718.
- Chapoval, S.P., Iijima, K., Marietta, E.V., Smart, M.K., Chapoval, A.I., Andrews, A.G., David, C.S., 2002. Allergic inflammatory response to short ragweed allergenic extract in HLA-DQ transgenic mice lacking CD4 gene. *J. Immunol.* 168, 890–899.
- Chung, K.F., 2014. Defining phenotypes in asthma: a step towards personalized medicine. *Drugs* 74, 719–728.
- Chung, K.F., Wenzel, S.E., Brozek, J.L., Bush, A., Castro, M., Sterk, P.J., Adcock, I.M., Bateman, E.D., Bel, E.H., Bleecker, E.R., Boulet, L.P., Brightling, C., Chanez, P., Dahlen, S.E., Djukanovic, R., Frey, U., Gaga, M., Gibson, P., Hamid, Q., Jajour, N.N., Mauad, T., Sorkness, R.L., Teague, W.G., 2014. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* 43, 343–373.
- Cohn, L., Homer, R.J., Marinov, A., Rankin, J., Bottomly, K., 1997. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *J. Exp. Med.* 186, 1737–1747.
- Conrad, M.L., Yildirim, A.O., Sonar, S.S., Kilic, A., Sudowe, S., Lunow, M., Teich, R., Renz, H., Garn, H., 2009. Comparison of adjuvant and adjuvant-free murine experimental asthma models. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 39, 1246–1254.
- Corren, J., Lemanske, R.F., Hanania, N.A., Korenblat, P.E., Parsey, M.V., Arron, J.R., Harris, J.M., Scheerens, H., Wu, L.C., Su, Z., Mosesova, S., Eisner, M.D., Bohen, S.P., Matthews, J.G., 2011. Lebrikizumab treatment in adults with asthma. *N. Engl. J. Med.* 365, 1088–1098.
- Daubeuf, F., Frossard, N., 2014. Eosinophils and the ovalbumin mouse model of asthma. *Methods Mol. Biol.* 1178, 283–293.
- De Luna, C.J., Moro, C.V., Guy, J.H., Zenner, L., Sparagano, O.A., 2009. Endosymbiotic bacteria living inside the poultry red mite (*Dermanyssus gallinae*). *Exp. Appl. Acarol.* 48, 105–113.
- DiGiovanni, F.A., Ellis, R., Wattie, J., Hirota, J.A., Southam, D.S., Inman, M.D., 2009. Concurrent dual allergen exposure and its effects on airway hyperresponsiveness, inflammation and remodeling in mice. *Dis. Models Mech.* 2, 275–282.
- Douwes, J., Zuidhof, A., Doekes, G., van der Zee, S.C., Wouters, I., Boezen, M.H., Brunekreef, B., 2000. (1-3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. *Am. J. Respir. Crit. Care Med.* 162 (134), 8–1354.
- Epstein, M.M., 2004. Do mouse models of allergic asthma mimic clinical disease? *Int. Arch. Allergy Immunol.* 133, 84–100.
- Epstein, M.M., 2006. Are mouse models of allergic asthma useful for testing novel therapeutics? *Exp. Toxicol. Pathol.: Off. J. Gesellschaft Toxikologische Pathologie* 57 (2), 41–44.
- Exley, C., Siesjo, P., Eriksson, H., 2010. The immunobiology of aluminium adjuvants: how do they really work? *Trends Immunol.* 31, 103–109.
- Expert Panel Report3(EPR-3), 2008. Guidelines for the Diagnosis and Management of Asthma – Summary Report 2007. *J. Allergy Clin. Immunol.* 121, 1330.
- Fan, T., Yang, M., Halayko, A., Mohapatra, S.S., Stephens, N.L., 1997. Airway responsiveness in two inbred strains of mouse disparate in IgE and IL-4 production. *Am. J. Respir. Cell Mol. Biol.* 17, 156–163.
- Fattouh, R., Pouladi, M.A., Alvarez, D., Johnson, J.R., Walker, T.D., Goncharova, S., Inman, M.D., Jordana, M., 2005. House dust mite facilitates ovalbumin-specific allergic sensitization and airway inflammation. *Am. J. Respir. Crit. Care Med.* 172, 314–321.
- Fuchs, B., Braun, A., 2008. Improved mouse models of allergy and allergic asthma—chances beyond ovalbumin. *Curr. Drug Targets* 9, 495–502.
- Gavino, A.C., Needham, G.R., High, W.A., 2008. Atopic dermatitis, patch testing, and house dust mites: a brief review. *Dermat. Contact Atopic Occup. Drug* 19, 121–128.
- Gregory, L.G., Causton, B., Murdoch, J.R., Mathie, S.A., O'Donnell, V., Thomas, C.P., Priest, F.M., Quint, D.J., Lloyd, C.M., 2009. Inhaled house dust mite induces pulmonary T helper 2 cytokine production. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 39, 1597–1610.
- Gregory, L.G., Lloyd, C.M., 2011. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol.* 32, 402–411.
- Guilbert, T.W., Denlinger, L.C., 2010. Role of infection in the development and exacerbation of asthma. *Expert Rev. Respir. Med.* 4, 71–83.
- Hamelmann, E., Takeda, K., Schwarze, J., Vella, A.T., Irvin, C.G., Gelfand, E.W., 1999. Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. *Am. J. Respir. Cell Mol. Biol.* 21, 480–489.
- Hammad, H., Chieppa, M., Perros, F., Willart, M.A., Germain, R.N., Lambrecht, B.N., 2009. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* 15, 410–416.
- Hansbro, P.M., Starkey, M.R., Kim, R.Y., Stevens, R.L., Foster, P.S., Horvat, J.C., 2012. Programming of the lung by early-life infection. *J. Dev. Orig. Health Dis.* 3, 153–158.
- Hatzivassiliou, M., Grainge, C., Kehagia, V., Lau, L., Howarth, P.H., 2010. The allergen specificity of the late asthmatic reaction. *Allergy* 65, 355–358.
- Herszberg, B., Ramos-Barbon, D., Tamaoka, M., Martin, J.G., Lavoie, J.P., 2006. Heaves, an asthma-like equine disease, involves airway smooth muscle remodeling. *J. Allergy Clin. Immunol.* 118, 382–388.
- Holgate, S.T., 2012. Innate and adaptive immune responses in asthma. *Nat. Med.* 18, 673–683.
- Holmes, A.M., Solari, R., Holgate, S.T., 2011. Animal models of asthma: value, limitations and opportunities for alternative approaches. *Drug Discov. Today* 16, 659–670.
- Jacquet, A., 2011. The role of the house dust mite-induced innate immunity in development of allergic response. *Int. Arch. Allergy Immunol.* 155, 95–105.
- Johnson, J.R., Wiley, R.E., Fattouh, R., Swirski, F.K., Gajewska, B.U., Coyle, A.J., Gutierrez-Ramos, J.C., Ellis, R., Inman, M.D., Jordana, M., 2004. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am. J. Respir. Crit. Care Med.* 169, 378–385.
- Jose, P.J., Griffiths-Johnson, D.A., Collins, P.D., Walsh, D.T., Moqbel, R., Totty, N.F., Truong, O., Hsuan, J.J., Williams, T.J., 1994. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.* 179, 881–887.
- Jungsuwadee, P., Benkovszky, M., Dekan, G., Stingl, G., Epstein, M.M., 2004. Repeated aerosol allergen exposure suppresses inflammation in B-cell-deficient mice with established allergic asthma. *Int. Arch. Allergy Immunol.* 133, 40–48.
- Karol, M.H., 1994. Animal models of occupational asthma. *Eur. Respir. J.* 7, 555–568.
- Kim, C.H., Ahn, J.H., Kim, S.J., Lee, S.Y., Kim, Y.K., Kim, K.H., Moon, H.S., Song, J.S., Park, S.H., Kwon, S.S., 2006. Co-administration of vaccination with DNA encoding T cell epitope on the Der p and BCG inhibited airway remodeling in

- a murine model of chronic asthma. *J. Asthma: Off. J. Assoc. Care Asthma* 43, 345–353.
- King, C., Brennan, S., Thompson, P.J., Stewart, G.A., 1998. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J. Immunol.* 161, 3645–3651.
- Koumoundouros, E., Bischof, R.J., Meeusen, E.N., Mareels, I.M., Snibson, K.J., 2006. Chronic airway disease: deteriorating pulmonary function in sheep associated with repeated challenges of house dust mite. *Exp. Lung Res.* 32, 321–330.
- Kumar, R.K., Foster, P.S., 2012. Are mouse models of asthma appropriate for investigating the pathogenesis of airway hyper-responsiveness? *Front. Physiol.* 3, 312.
- Kumar, R.K., Herbert, C., Foster, P.S., 2008. The “classical” ovalbumin challenge model of asthma in mice. *Curr. Drug Targets* 9, 485–494.
- Kumar, R.K., Herbert, C., Yang, M., Koskinen, A.M., McKenzie, A.N., Foster, P.S., 2002. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 32, 1104–1111.
- Lambert, A.L., Winsett, D.W., Costa, D.L., Selgrade, M.K., Gilmour, M.I., 1998. Transfer of allergic airway responses with serum and lymphocytes from rats sensitized to dust mite. *Am. J. Respir. Crit. Care Med.* 157, 1991–1999.
- Leclerc, M., Lavoie-Lamoureux, A., Lavoie, J.P., 2011. Heaves, an asthma-like disease of horses. *Respirology* 16, 1027–1046.
- Leong, K.P., Huston, D.P., 2001. Understanding the pathogenesis of allergic asthma using mouse models. *Ann. Allergy Asthma Immunol.: Off. Publ. Am. Coll. Allergy Asthma Immunol.* 87, 96–109.
- Lloyd, C.M., 2007. Building better mouse models of asthma. *Curr. Allergy Asthma Rep.* 7, 231–236.
- Mahmutovic-Persson, I., Akbarshahi, H., Bartlett, N.W., Glanville, N., Johnston, S.L., Brandelius, A., Uller, L., 2014. Inhaled dsRNA and rhinovirus evoke neutrophilic exacerbation and lung expression of thymic stromal lymphopoietin in allergic mice with established experimental asthma. *Allergy* 69, 348–358.
- Manni, M.L., Trudeau, J.B., Scheller, E.V., Mandalapu, S., Elloso, M.M., Kolls, J.K., Wenzel, S.E., Alcorn, J.F., 2014. The complex relationship between inflammation and lung function in severe asthma. *Mucosal Immunol.* 7, 1186–1198.
- Martin, R.A., Hodgkins, S.R., Dixon, A.E., Poynter, M.E., 2014. Aligning mouse models of asthma to human endotypes of disease. *Respirology* 19, 823–833.
- Maunsell, K., Wraith, D.G., Cunningham, A.M., 1968. Mites and house-dust allergy in bronchial asthma. *Lancet* 1, 1267–1270.
- McMillan, S.J., Lloyd, C.M., 2004. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 34, 497–507.
- Mercer, P.F., Abbott-Banner, K., Adcock, I.M., Knowles, R.G., 2015. Translational models of lung disease. *Clin. Sci.* 128, 235–256.
- Mori, H., Parker, N.S., Rodrigues, D., Hulland, K., Chappell, D., Hincks, J.S., Bright, H., Evans, S.M., Lamb, D.J., 2013. Differences in respiratory syncytial virus and influenza infection in a house-dust-mite-induced asthma mouse model: consequences for steroid sensitivity. *Clin. Sci.* 125, 565–574.
- Mullane, K., 2011. Asthma translational medicine: report card. *Biochem. Pharmacol.* 82, 567–585.
- Mullane, K., Williams, M., 2014. Animal models of asthma: reprise or reboot? *Biochem. Pharmacol.* 87, 131–139.
- Muller, K.M., Jaunin, F., Masouye, I., Saurat, J.H., Hauser, C., 1993. Th2 cells mediate IL-4-dependent local tissue inflammation. *J. Immunol.* 150, 5576–5584.
- Nelson Jr., R.P., DiNicola, R., Fernandez-Caldas, E., Seleznick, M.J., Lockey, R.F., Good, R.A., 1996. Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in nonasthmatic control subjects. *J. Allergy Clin. Immunol.* 98, 258–263.
- Nials, A.T., Uddin, S., 2008. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis. Models Mech.* 1, 213–220.
- Padrid, P., 1992. Chronic lower airway disease in the dog and cat. *Probl. Vet. Med.* 4, 320–344.
- Persson, C., 2014. Simvastatin inhibits smoke-induced airway epithelial injury: implications for COPD therapy. *Eur. Respir. J.* 43, 1208–1211.
- Persson, C., Uller, L., 2014. Theirs but to die and do: primary lysis of eosinophils and free eosinophil granules in asthma. *Am. J. Respir. Crit. Care Med.* 189, 628–633.
- Persson, C.G., 2002. Con: mice are not a good model of human airway disease. *Am. J. Respir. Crit. Care Med.* 166, 6–7; discussion 8.
- Phipps, S., Lam, C.E., Kaiko, G.E., Foo, S.Y., Collison, A., Mattes, J., Barry, J., Davidson, S., Oreo, K., Smith, L., Mansell, A., Matthaei, K.I., Foster, P.S., 2009. Toll/IL-1 signaling is critical for house dust mite-specific helper T cell type 2 and type 17 [corrected] responses. *Am. J. Respir. Crit. Care Med.* 179, 883–893.
- Platt-Mills, T.A., Sporik, R.B., Chapman, M.D., Heymann, P.W., 1995. The role of indoor allergens in asthma. *Allergy* 50, 5–12.
- Ressmeyer, A.R., Larsson, A.K., Vollmer, E., Dahlen, S.E., Uhlig, S., Martin, C., 2006. Characterisation of guinea pig precision-cut lung slices: comparison with human tissues. *Eur. Respir. J.* 28, 603–611.
- Ricciardolo, F.L., Nijkamp, F., De Rose, V., Folkerts, G., 2008. The guinea pig as an animal model for asthma. *Curr. Drug Targets* 9, 452–465.
- Roche, N., Chinnet, T.C., Huchon, G.J., 1997. Allergic and nonallergic interactions between house dust mite allergens and airway mucosa. *Eur. Respir. J.* 10, 719–726.
- Rochlitzer, S., Hoymann, H.G., Muller, M., Braun, A., 2014. No exacerbation but impaired anti-viral mechanisms in a rhinovirus-chronic allergic asthma mouse model. *Clin. Sci.* 126, 55–65.
- Sarpong, S.B., Zhang, L.Y., Kleeberger, S.R., 2003. A novel mouse model of experimental asthma. *Int. Arch. Allergy Immunol.* 132, 346–354.
- Schelegle, E.S., Gershwin, L.J., Miller, L.A., Fanucchi, M.V., Van Winkle, L.S., Gerriets, J.P., Walby, W.F., Omlor, A.M., Buckpitt, A.R., Tarkington, B.K., Wong, V.J., Joad, J.P., Pinkerton, K.B., Wu, R., Evans, M.J., Hyde, D.M., Plopper, C.G., 2001. Allergic asthma induced in rhesus monkeys by house dust mite (*Dermatophagoides farinae*). *Am. J. Pathol.* 158, 333–341.
- Shardonofsky, F.R., Venzor 3rd, J., Barrios, R., Leong, K.P., Huston, D.P., 1999. Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. *J. Allergy Clin. Immunol.* 104, 215–221.
- Shifren, A., Witt, C., Christie, C., Castro, M., 2012. Mechanisms of remodeling in asthmatic airways. *J. Allergy* 2012, 316049.
- Shin, Y.S., Takeda, K., Gelfand, E.W., 2009. Understanding asthma using animal models. *Allergy Asthma Immunol. Res.* 1, 10–18.
- Shinagawa, K., Kojima, M., 2003. Mouse model of airway remodeling: strain differences. *Am. J. Respir. Crit. Care Med.* 168, 959–967.
- Snibson, K.J., Bischof, R.J., Slocombe, R.F., Meeusen, E.N., 2005. Airway remodelling and inflammation in sheep lungs after chronic airway challenge with house dust mite. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 35, 146–152.
- Starkey, M.R., Jarnicki, A.G., Essilfie, A.T., Gellatly, S.L., Kim, R.Y., Brown, A.C., Foster, P.S., Horvat, J.C., Hansbro, P.M., 2013. Murine models of infectious exacerbations of airway inflammation. *Curr. Opin. Pharmacol.* 13, 337–344.
- Stevenson, C.S., Birrell, M.A., 2011. Moving towards a new generation of animal models for asthma and COPD with improved clinical relevance. *Pharmacol. Ther.* 130, 93–105.
- Sun, G., Stacey, M.A., Schmidt, M., Mori, L., Mattoli, S., 2001. Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J. Immunol.* 167, 1014–1021.
- Takeda, K., Gelfand, E.W., 2009. Mouse models of allergic diseases. *Curr. Opin. Immunol.* 21, 660–665.
- Thomas, W.R., Smith, W.A., Hales, B.J., Mills, K.L., O'Brien, R.M., 2002. Characterization and immunobiology of house dust mite allergens. *Int. Arch. Allergy Immunol.* 129, 1–18.
- Trompette, A., Divanovic, S., Visintin, A., Blanchard, C., Hegde, R.S., Madan, R., Thorne, P.S., Wills-Karp, M., Gioannini, T.L., Weiss, J.P., Karp, C.L., 2009. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 457, 585–588.
- Uller, L., Leino, M., Bedke, N., Sammut, D., Green, B., Lau, L., Howarth, P.H., Holgate, S.T., Davies, D.E., 2010. Double-stranded RNA induces disproportionate expression of thymic stromal lymphopoietin versus interferon-beta in bronchial epithelial cells from donors with asthma. *Thorax* 65, 626–632.
- Uller, L., Mathiesen, J.M., Alenmyr, L., Korsgren, M., Ulven, T., Hogberg, T., Andersson, G., Persson, C.G., Kostenis, E., 2007. Antagonism of the prostaglandin D2 receptor CRTH2 attenuates asthma pathology in mouse eosinophilic airway inflammation. *Respir. Res.* 8, 16.
- Uller, L., Rydell-Tormanen, K., Persson, C.G., Erjefelt, J.S., 2005. Anti-Fas mAb-induced apoptosis and cytolysis of airway tissue eosinophils aggravates rather than resolves established inflammation. *Respir. Res.* 6, 90.
- Valerio, C.R., Murray, P., Arlian, L.G., Slater, J.E., 2005. Bacterial 16 S ribosomal DNA in house dust mite cultures. *J. Allergy Clin. Immunol.* 116, 1296–1300.
- van Schayck, O.C.P., 2013. Global strategies for reducing the burden from asthma a report of a lecture given at The University of Edinburgh, Edinburgh, UK, October 1st 2012. *Prim. Care Respir. J.* 22, 239–243.
- Walker, C., Checkel, J., Cammisuli, S., Leibson, P.J., Gleich, G.J., 1998. IL-5 production by NK cells contributes to eosinophilic infiltration in a mouse model of allergic inflammation. *J. Immunol.* 161, 1962–1969.
- Wark, P.A., Johnston, S.L., Moric, I., Simpson, J.L., Hensley, M.J., Gibson, P.G., 2002. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur. Respir. J.* 19, 68–75.
- Warner, R.L., Lukacs, N.W., Shapiro, S.D., Bhagavathula, N., Nerusu, K.C., Varani, J., Johnson, K.J., 2004. Role of metalloelastase in a model of allergic lung responses induced by cockroach allergen. *Am. J. Pathol.* 165, 1921–1930.
- Webb, D.C., Mahalingam, S., Cai, Y., Matthaei, K.I., Donaldson, D.D., Foster, P.S., 2003. Antigen-specific production of interleukin (IL)-13 and IL-5 cooperate to mediate IL-4/Ralpha-independent airway hyperreactivity. *Eur. J. Immunol.* 33, 3377–3385.
- Wegmann, M., 2008. Animal models of chronic experimental asthma – strategies for the identification of new therapeutic targets. *J. Occup. Med. Toxicol.* 3 (1), S4.
- Wegmann, M., Hauber, H.P., 2010. Experimental approaches towards allergic asthma therapy-murine asthma models. *Recent Pat. Inflamm. Allergy Drug Discov.* 4, 37–53.
- Weinberg, E.G., 2011. The Wao white book on allergy 2011–2012. *Curr. Allergy Clin. Immunol.* 24, 156–157.
- Wenzel, S., Holgate, S.T., 2006. The mouse trap: it still yields few answers in asthma. *Am. J. Respir. Crit. Care Med.* 174, 1173–1176; discussion 1176–1178.
- Yawn, B.P., 2008. Factors accounting for asthma variability: achieving optimal symptom control for individual patients. *Prim. Care Respir. J.: J. Gen. Pract. Airw. Group* 17, 138–147.
- Zosky, G.R., Sly, P.D., 2007. Animal models of asthma. *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 37, 973–988.