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Translational value of animal models of asthma: Challenges and promises

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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.

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 mouldy hay horses can develop heaves as a naturally occurring neutrophil dominated obstructive pulmonary disease (Herszberg et al., 2006; Leclere et al., 2011). Similar to human asthma, heaves is further characterised by chronic airway inflammation, airway hyperresponsiveness (AHR) and reversible bronchoconstriction (Leclere 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

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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 (Anon, 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 subepithelial 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 sensitisations 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 intransally (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.,

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