

The "Classical" Ovalbumin Challenge Model of Asthma in Mice

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Abstract: Ovalbumin challenge models of asthma offer many opportunities for increasing our understanding of the pathogenetic mechanisms underlying this disease, as well as for identifying novel therapeutic targets. There is no single "classical" model, because numerous alternatives exist with respect to the choice of mouse strain, method of sensitisation, route and duration of challenge, and approach to assessing the host response. Moreover, the limitations of these models need to be recognised when attempting to interpret experimental findings. Nevertheless, careful use of well-defined models allows investigators to answer specific questions that are otherwise difficult to address.

Key Words: Asthma, animal models, airway inflammation, airway hyper-responsiveness, airway remodelling.

1. INTRODUCTION

At the time of writing, a PubMed search for:

Mouse AND ovalbumin AND (asthma OR allergy)

Yielded just over 2000 references. Viewed in isolation, this statistic might justify designating ovalbumin challenge-induced airway inflammation as the "classical" mouse model of asthma. In reality, however, whether there actually is a classical model is open to debate, because there are many sources of variation in the host response in such experimental systems. Moreover, no single model can adequately reproduce the spatial and temporal recruitment of the various cell types involved in the different phases of clinical asthma. Despite the pitfalls associated with modelling allergic asthma in mice, we nevertheless contend that such investigations provide many opportunities for increasing our understanding of pathogenetic mechanisms of disease [1]. In this review, we will discuss the spectrum of allergic bronchopulmonary disease in mice following ovalbumin challenge, as well as the usefulness and limitations of such models.

SOURCES OF VARIATION IN OVALBUMIN CHALLENGE MODELS

A novice attempting to come to grips with the literature on modelling allergic asthma in mice is likely to be both bemused and bewildered by the variety of models that different investigators have employed. Even focusing solely on ovalbumin challenge, there is a spectrum of models, because the choice of mouse strain, method of sensitisation, as well as the route, dose and duration of challenge all influence the nature of the inflammatory and other lesions that develop.

Strain

The choice of mouse strain directly affects the pattern of allergic inflammation elicited in ovalbumin challenge models of asthma. While sometimes considered to be related to the predisposition of the strain to develop a Th2-biased immunological response and/or to produce IgE, this is not necessarily the case. Moreover, although mice of the BALB/c strain are widely regarded as being more Th2-biased than other strains such as C57BL/6, this concept is primarily based on the differential susceptibility of these strains to parasitic infection e.g. with *Leishmania major* [2]. In fact C57BL/6 mice mount robust Th2 responses and in studies of sensitised mice challenged with ovalbumin aerosol, eosinophil recruitment as assessed in bronchoalveolar lavage (BAL) fluid was greater in C57BL/6 and 129/SvIm mice than in A/J and BALB/c mice [3, 4]. However, BALB/c mice produce higher levels of anti-ovalbumin IgE antibody [3]. Furthermore, the concentration of Th2 cytokines such as IL-4 and IL-5 in BAL fluid is greater in BALB/c than in C57BL/6 mice [5].

Strain dependency of airway hyper-responsiveness (AHR) in ovalbumin challenge models has been well documented. For example, in a comparison of 12 mouse strains, airway responsiveness to methacholine was greatest in AKR and SWR mice but virtually absent in A/J mice [3]. Such differences may at least in part be related to polymorphisms of the IL-4 receptor [6]. However, AHR results are in turn influenced by methods of sensitisation and challenge, because A/J mice exhibited marked AHR after repeated intranasal challenge, whereas C57BL/6 mice did not [7].

In chronic challenge models, development of the changes of airway wall remodelling is also dependent on the strain used. Shinagawa *et al.* found that their model of chronic intranasal challenge, A/J mice exhibited more marked changes than BALB/c mice, which were in turn greater than those in C57BL/6 animals [7]. In our own studies, subepithelial

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fibrosis was reproducibly elicited in BALB/c mice chronically challenged by inhalation of low mass concentrations of ovalbumin aerosol, but was essentially absent in C57BL/6 mice [1].

Method of Sensitisation

The dose of antigen used for sensitisation and the route of administration are both important variables between models. For intraperitoneal sensitisation of mice, the dose of ovalbumin used varies from as little as 1 µg [8] to as much as 8000 µg per animal [9]. However, doses of between 10 and 50 µg of ovalbumin are most commonly employed. In at least one study, levels of IgE and the severity of pulmonary inflammation and eosinophil accumulation in BAL fluid in response to challenge with ovalbumin aerosol were found to be inversely related to the sensitising dose [10]. Moreover, AHR to methacholine was demonstrated in mice sensitised to 10 µg, but not in mice sensitised to 1000 µg of ovalbumin.

Sensitisation is most often achieved by multiple intraperitoneal injections of antigen [4, 11]. Mice can also be sensitised to ovalbumin by subcutaneous [12, 13], intranasal [7, 14] or intratracheal [15, 16] delivery. Whether effective sensitisation can reliably be achieved by inhalational challenge alone is less clear, although this approach has been reported to be successful [17]. The route of sensitisation clearly does influence the host response; in animals sensitised by intraperitoneal injection, IgE production is typically greater than in animals sensitised intranasally [18, 19].

Another potential source of differing results between models is the use of adjuvants, which are intended to boost the immune response and to promote the production of Th2 cytokines [20]. Potassium aluminium sulfate (alum) is the most commonly used adjuvant, with ovalbumin typically being adsorbed to between 1 mg [21] and 40 mg [10] of alum for intraperitoneal injection. Two injections of ovalbumin with adjuvant, 7 to 14 days apart, usually induce a strong Th2-biased immune response with high levels of total and antigen-specific IgE. Adjuvant-free protocols require a greater number of injections to achieve sensitisation [12, 22]. However, the use of an adjuvant during sensitisation may significantly alter the response to subsequent allergen challenge. Recent studies indicate that whereas AHR and airway inflammation are independent of mast cells when animals are sensitised with ovalbumin-alum, these responses are mast cell-dependent when an alum-free protocol is used [23].

Route and Duration of Challenge

There are numerous options with respect to challenge protocols, which in combination with the choice of strain and different approaches to sensitisation lead to a enormous diversity of models [24]. Some of the resultant possibilities are illustrated in Tables 1 and 2. The route and duration of challenge are perhaps the most important variables in "classical" models of allergic asthma.

Animals can be challenged by inhalation of aerosolised ovalbumin, by intratracheal administration or by intranasal

Table 1. Examples of Short-Term Ovalbumin Challenge Models of Asthma in Mice

Strain	Sensitisation Method	Challenge Route	Challenge Protocol	Key Features	Ref.
BALB/c	-	Inhalation	10 × daily 20 min aerosol	Enhanced response of tracheal smooth muscle to methacholine or electrical stimulation <i>in vitro</i> .	[25]
C57BL/6	Ova/Alum (i.p) day 0	Inhalation	7 × daily 30 min aerosol days 14 – 21	Peribronchial inflammation, eosinophils and lymphocytes in BAL fluid.	[26]
B6D2F1/J	Ova/Alum (i.p) days 0 and 5	Inhalation	2 × 60 min aerosol on day 12	Elevated IgE, severe peribronchiolar and perivascular eosinophil accumulation; epithelial damage.	[27]
C57BL/6	Ova/Alum (i.p) days 0 and 12	Inhalation	3 × 30 min day 24, 1 × 30 min day 26, 28, 30, 32	AHR and an increase in peribronchiolar and perivascular eosinophils; epithelial shedding and mucosal oedema.	[28]
BALB/c	7 × Ovalbumin (i.p) alternate days	Intratracheal	3 × 20 µg antigen every third day	Inflammation in airways and lung tissue; goblet cell hyperplasia and epithelial hypertrophy; early subepithelial fibrosis.	[29]
BALB/c	7 × Ovalbumin (i.p) alternate days	Inhalation	8 × daily 5 min aerosol	AHR to methacholine challenge (decreased by anti-IFN-γ); eosinophil accumulation in BAL fluid (decreased by anti-IL-5).	[30]
BALB/c	Ova/Alum (i.p) days 0 and 7	Intranasal	1 × 50 µl 1.5% ovalbumin solution	AHR to acetylcholine; eosinophils, lymphocytes and cytokines (IL-4, IL-5 and IFN-γ) increased in BAL fluid.	[31]
BALB/c	Ova/Alum (i.p) days 0 and 14	Inhalation	20 min aerosol days 28-30 (1%) and 32 (5%)	Allergen-induced early and late phase bronchoconstriction.	[32]

Table 2. Examples of Chronic Ovalbumin Challenge Models of Asthma in Mice

Strain	Sensitisation Method	Challenge Route	Challenge Protocol	Key Features	Ref.
BALB/c	Ova/Alum (i.p) days 0 and 14	Inhalation	30 min aerosol 3 days/ week × 6 weeks	Acute on chronic airway inflammation; AHR from central airways, subepithelial fibrosis, epithelial cell hypertrophy; all sustained.	[33]
C57BL/6	Ova/Alum (i.p) days 0, 7 and 14	Inhalation (nose only)	60 min aerosol 5 days/week × 6 weeks	Inflammatory cells in BAL fluid; increased IgE and AHR after 3 weeks; all significantly diminished after 6 weeks.	[34]
BALB/c	Ova/Alum (i.p) days 0, 7 and 14	Inhalation	2 days/week for 4 weeks	Inflammatory cells in BAL fluid, increased IgE, AHR, mucous cell hyperplasia and epithelial thickening	[35]
C57BL/6	Ova/Alum (i.p) days 0 and 14	Inhalation	30 min aerosol 3 days/week × 6 weeks	AHR, subepithelial fibrosis and extensive peribronchial inflammation; eosinophils and lymphocytes increased in BAL fluid.	[36]
BALB/c	Ova/Alum (i.p) days 0 and 14	Inhalation	60 min aerosol 3 days/ week × (up to) 8 weeks	AHR to methacholine, subepithelial fibrosis and goblet cell hyperplasia; inflammation suppressed by 8 weeks.	[37]
A/J	-	Intranasal	3 days/week for up to 12 weeks	Persistent pulmonary inflammation and AHR to methacholine (i.v); increased goblet cells and airway smooth muscle, collagen deposition.	[7]
C57BL/6	Ova/Alum (i.p.) days 0 and 7	Inhalation	15 min aerosol 3/day, 3 days/week for 8 weeks	Eosinophilic inflammation at 1 week, downregulated by 8 weeks; epithelial thickening, peribronchiolar fibrosis and smooth muscle hyperplasia, borderline AHR	[38]
BALB/c	Ova/Alum (i.p) days 0 and 12	Inhalation	20 min, days 18-23 + 3 days/week until day 55	AHR, subepithelial fibrosis, smooth muscle and goblet cell hyperplasia; IL-4 and IFN- γ increased in lung tissue	[39]
BALB/c	Ova/Alum (i.p) days 0, 14 and 21	Inhalation	2 days/week for 12 weeks	Persistent pulmonary inflammation and AHR to methacholine; initially high levels of BAL cytokines declined by 12 weeks	[40]

instillation. Inhalational challenge typically involves placing mice in a chamber into which an aerosol of ovalbumin is delivered. Exposure chambers vary from simple Plexiglas boxes to sophisticated apparatus in which airflow through the chamber can be monitored and regulated. This method of antigen challenge is popular, as aerosols with a constant particle size can easily be generated using ultrasonic or compressed air-driven nebulisers. It has been argued that inhalation of aerosolised antigen more closely resembles allergen exposure in patients with asthma and is therefore more relevant than other challenge routes. Moreover, in most inhalational challenge models, mice remain conscious throughout the procedure and do not need to be restrained, which reduces stress to the animals.

Most inhalational challenge models are short-term, in that mice are exposed to one or several challenges over 4-8 consecutive days, which results in eosinophil accumulation and AHR. Such models are usually associated with marked perivascular and parenchymal inflammation in the lungs, which are not features of human asthma [41]. These appearances are likely to be a consequence of the unregulated delivery of aerosolised antigen in these protocols, which leads to very high mass concentrations of aerosol being delivered into the exposure chamber. In studies of the dose-response relationship in silica-induced pulmonary fibrosis [42], we showed that development of progressive parenchymal lesions depended on exceeding a mass concentration

threshold of airborne particles. High-level aerosol exposures are likely to similarly overwhelm mechanisms for clearance of antigen from the airways, leading to severe pulmonary inflammation.

Chronic challenge models of asthma in mice involve repeated exposure to antigen for up to 8 weeks. However, long term challenge with high mass concentrations of antigen often leads either to induction of a granulomatous inflammatory response [43], or to down-regulation of inflammation, AHR and structural airway changes of asthma [34, 44, 45]. Again, differences in protocols can have a significant effect on the study outcomes e.g. Sakai *et al.* reported that inflammation but not remodelling are inhibited by long term exposure to ovalbumin aerosol [46]. Nevertheless, because the development of tolerance reduces the inflammatory response to repeated antigen challenges, ovalbumin inhalational challenge models are sometimes portrayed as unsuitable for assessment of the chronic lesions of asthma.

We hypothesised that the development of tolerance in these models was related to the high mass concentrations of aerosolised allergen used, which would have overwhelmed respiratory clearance mechanisms. To avoid the down-regulation of airway responses during chronic challenge, we carefully controlled the mass concentrations of aerosol at very low levels [33]. Chronic exposure of sensitised mice to a mass concentration of 3 mg/m³ of aerosolised ovalbumin, 30 min/day on 3 days/week for 6-8 weeks, resulted in mild

acute-on-chronic inflammation of the airway wall, progressive remodelling of the airways and persistent AHR (Fig. 1). Pulmonary inflammation was absent and AHR was demonstrated to originate from the conducting airways rather than the lung parenchyma. These changes closely resembled the structural and functional abnormalities of chronic human asthma [1, 33].

More recently, we have established a model of an allergen-induced acute exacerbation of chronic asthma, which further emphasises the critical importance of the mass concentration of aerosol used for challenge. Background lesions of mild asthma were induced by chronic low-level challenge for 4 weeks, after which animals were exposed for a 30 min period to a single moderate-level challenge with 30 mg/m³ of aerosolised antigen (Fig. 2). This is still a much lower mass concentration than is used by most investigators in models of allergic airway inflammation, because 10-100 fold higher concentrations are typical in uncontrolled exposure chambers. Nevertheless, this moderate-level challenge is associ-

ated with more marked airway inflammation which extends further distally than in the chronic challenge model, together with a pattern of AHR distinct from that seen in the chronic challenge model, reflecting the distal airway involvement [47]. Compared to high-level challenge models, however, the inflammatory response in our model of an acute exacerbation remains mild and the numbers of eosinophils, lymphocytes and neutrophils in BAL fluid are still relatively modest. Thus the features of this model are very similar to those of a clinical exacerbation of asthma. Table 3 compares the human disease with high-level challenge, short-term models and the chronic and acute exacerbation models we have developed.

ASSESSING THE "ASTHMATIC" RESPONSE

In evaluating the data from studies in ovalbumin challenge models of asthma, another significant set of issues relates to the approaches and techniques used for assessing a response. Again, there are numerous alternatives, not all of which parallel assessment in human asthma.

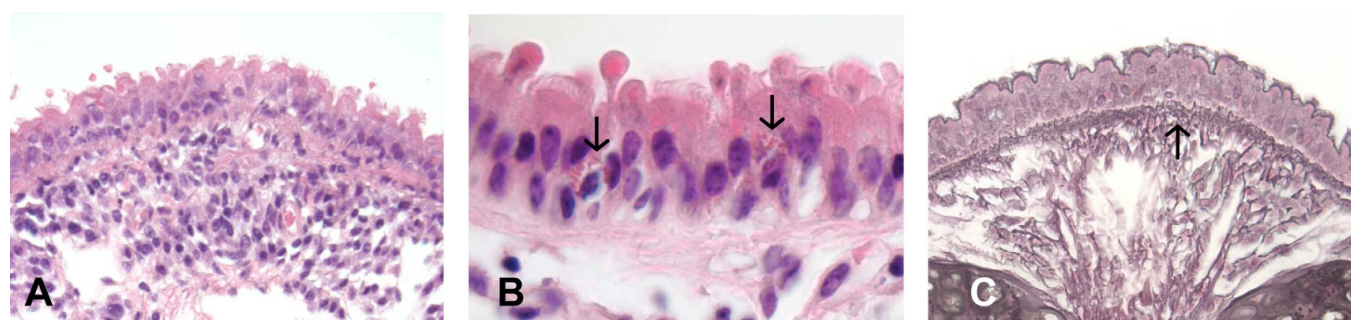


Fig. (1). Hallmark lesions of human asthma develop in 100% of animals in the chronic challenge model, including chronic inflammation in the lamina propria of the airway wall (A), intraepithelial accumulation of eosinophils (arrows, B) and subepithelial fibrosis (arrow, C).

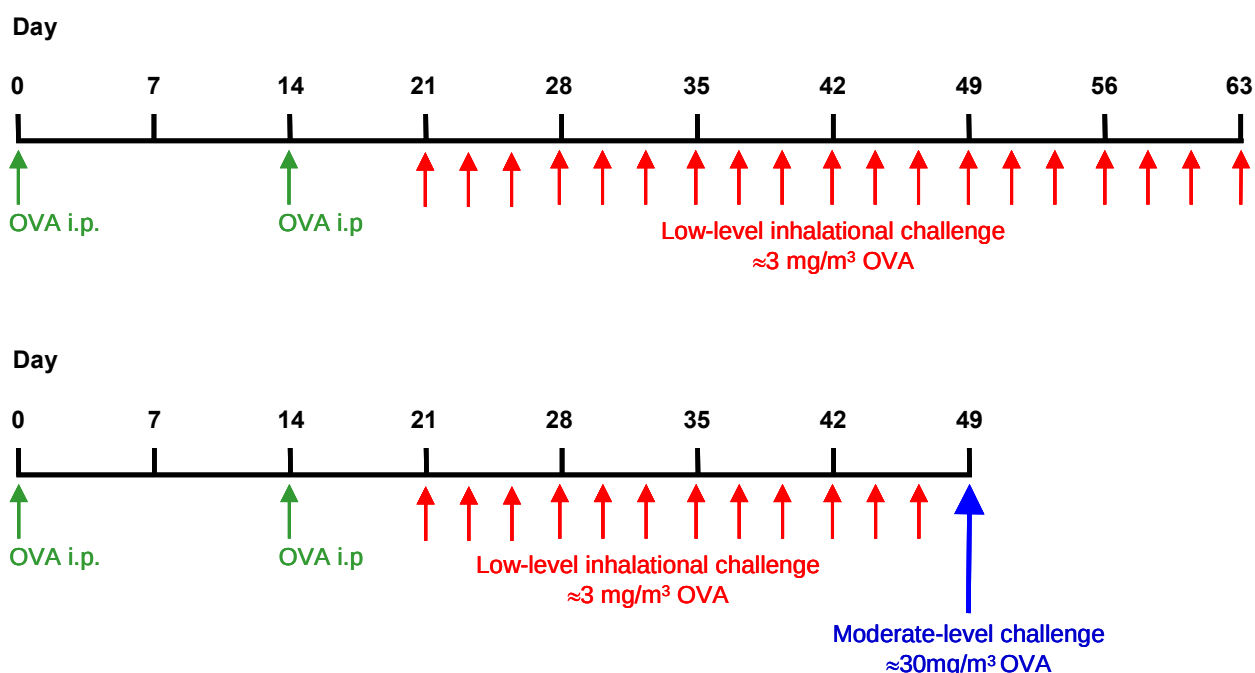


Fig. (2). The chronic challenge (above) and acute exacerbation (below) models of asthma employed in our laboratories.

Table 3. Comparison of Human Asthma with Various Ovalbumin Challenge Models

Feature	Human (Exacerbation)	Mouse (Short-Term Models) ^a	Mouse (Chronic Model) ^b	Mouse (Exacerbation) ^c
Distal airway inflammation	+++	++++	—	++
Peak eosinophil recruitment	?	24 h	4 h	4 h
Eosinophils in BAL fluid	++	++++	—	+
Lymphocytes in BAL fluid	++	+	++	+++
"Peripheral" AHR	+	+	—	+
Chronic inflammation in tissue	+++	—	+++	+++
Airway wall remodelling	+++	—	+++	+++

^aHigh-level challenge, short-term models such as in Table 1.^bModel of low-level, chronic challenge [33].^cModel of an acute exacerbation of chronic asthma [47].

Inflammation

A common approach is to quantify total and differential cell counts in BAL fluid. While this is experimentally convenient, it is worth emphasising that the increases in number and percentage of eosinophils in short-term high-level challenge models are far higher than ever seen in patients with asthma. Whereas eosinophils typically account for only 1-3% of cells obtained by bronchoalveolar lavage in human asthmatics, they comprise 40-80% of the recovered cells in short-term ovalbumin challenge models [48]. In models employing low-level chronic challenge, such as that developed in our laboratories, the total number of BAL cells does not increase significantly following exposure to antigen and, except for a small increase in the proportion of lymphocytes, there is little evidence of inflammatory cell recruitment [49]. In contrast, assessment of the mild inflammatory response in tissue sections is much more reliable, yielding consistent data both in terms of recruitment of eosinophils into the airway epithelium (which is a typical feature of human asthma [50]) and of accumulation of lymphocytes, plasma cells and macrophages in the lamina propria of the large airways. A significant point in this regard is that assessment must be performed at the appropriate anatomical site. For example, it has been asserted that intraepithelial accumulation of eosinophils does not occur in short-term models [51] but this claim is based on examination of intrapulmonary airways, where Clara cells are the predominant cell type. We have shown that eosinophils are readily detected in the airway epithelium of the trachea of sensitised mice after a single high-level challenge [52], reflecting the similarity between the tracheal epithelium in mice and bronchial epithelium in humans [53].

Comparable arguments apply to the assessment of expression of inflammatory mediators/cytokines in ovalbumin challenge models. While marked elevations of cytokine levels in BAL fluid and large fold changes in mRNA expression in tissues are demonstrable in short-term high-level challenge models, more is not necessarily better in terms of replicating features of the human disease. Because the focus of such investigations should be on expression of mediators involved in airway inflammation, it follows that it would be

logical to examine such expression in the airway walls themselves. A useful approach for selectively sampling airway tissues is the blunt dissection method developed by Kenyon *et al.* [37], which we have successfully employed to demonstrate the relatively small magnitude increases in inflammatory cytokine expression in the airways in our low-level chronic challenge model of asthma [37a].

Airway Remodelling

In human asthma, the structural changes of airway remodelling include subepithelial fibrosis, smooth muscle hypertrophy and/or hyperplasia, epithelial proliferation and mucous cell hyperplasia/metaplasia, as well as increased vascularity of the mucosa [54]. Ovalbumin challenge models can replicate some of these features of chronic asthma, although it is worth noting that goblet cell change is in fact a very rapidly developing response [55]. In high-level chronic challenge models, the extensive parenchymal inflammation progresses to development of fibrosis surrounding small intrapulmonary airways. This peribronchiolar scarring is not synonymous with subepithelial fibrosis, despite the widespread misuse of the terminology by investigators. It is more appropriate to refer to such changes as airway fibrosis [37]. However, lesions that closely resemble classical subepithelial fibrosis in asthmatics are observed in the trachea of mice in the low-level chronic challenge model employed in our laboratories [33].

Interestingly, only high-level challenge models of chronic ovalbumin exposure appear to elicit hypertrophy of airway smooth muscle in mice [7, 39, 56-58]. No such changes are observed in our low-level chronic challenge model.

Airway Hyper-Responsiveness

This area is plagued by controversy. To some extent, this is hardly surprising, given the fundamental differences between assessing AHR in patients, which is based on voluntary forced expiration, and in mice, which usually relies on invasive measurements in anaesthetised ventilated animals. In any case, AHR to methacholine in mice may primarily be related to responses other than smooth muscle/myofibroblast contraction e.g. early airway closure as a consequence of

thickening of the airway walls, or surfactant inactivation as a consequence of inflammatory exudation [59-61]. Thus, it remains unclear whether assessing changes in parameters such as transpulmonary resistance or dynamic compliance in mouse models is primarily an indirect measure of airway and/or parenchymal inflammation, rather than an independent index of physiological changes. Moreover, results appear to be highly dependent on whether the cholinergic stimulus is administered by inhalation or intravenous injection, with the latter apparently provoking a much more marked response in ovalbumin challenge models [62].

Various alternative approaches for assessing AHR have been reported. Non-invasive methods based on single- or dual-chamber plethysmography appear to have much merit [63, 64]. A whole-body plethysmography technique which gained considerable popularity, because it can be performed in unanaesthetised and unrestrained mice, is the measurement of the dimensionless parameter known as enhanced Pause (correctly abbreviated P_{enh} but widely and inaccurately referred to as $penH$) [65]. Unfortunately this technique has limitations in models associated with extensive parenchymal disease, in which abnormalities may correlate poorly with airway and tissue mechanical properties [66]. The method has therefore been the subject of extensive criticism [67-69] and has fallen into disfavour, even though many of the criticisms can at least in part be answered [70]. Nevertheless, P_{enh} may still have value as a screening procedure and certainly proved useful for assessing AHR in our low-level chronic challenge model, in which parenchymal disease is not a confounding factor.

Other methods such as the forced oscillation technique are considered to be more reliable for assessing AHR in mice [71, 72]. A recent addition to the range of available methods is measurement of the decrease in tidal midexpiratory flow (EF50), for which there are both noninvasive and invasive implementations [73-75]. This may be a particularly efficient approach to repeatable assessment of AHR.

USEFULNESS OF OVALBUMIN CHALLENGE MODELS

Having noted the cautions and criticisms above, the reader may wonder whether models of allergic airway inflammation based on ovalbumin challenge have any real use. They most certainly do and will continue to be valuable for understanding pathogenesis and investigating potential therapeutic targets in asthma. Of course, the models are open to criticism. The airways of mice are fundamentally different from humans in that they exhibit a racemose rather than a binary branching pattern, with far fewer orders of branching. The large diameter of the airways in relation to body size may well explain why mice do not develop "symptoms" of asthma [76]. It can also be argued that ovalbumin is not an allergen to which humans naturally become sensitised. However, that is not the point. Models are just that – models – and it is unsound to claim that because mouse models do not fully replicate asthma in humans, they are not able to provide insights into the pathogenesis of the disease [77, 78]. Every experimental model has its strengths and weaknesses [79]. In the case of ovalbumin challenge, for example, the use of a relatively defined, widely available antigen which can be

administered in controlled dosage is experimentally advantageous. In the remainder of this section, we highlight some significant observations that have been made using ovalbumin challenge models of asthma in mice, and identify some promising directions for future research.

Understanding Pathogenesis

The use of ovalbumin challenge models of asthma has significantly increased our understanding of the basic mechanisms of allergic inflammation and the underlying immunological response, allowing investigators to address specific questions that are difficult to answer in patients. The key regulatory role of $CD4^+$ T-lymphocytes in asthmatic inflammation and the importance of Th2 cytokines have been highlighted by numerous studies in mice [80-82]. In particular, studies on IL-5 [8, 28, 82, 83] and IL-13 [84-89] in such models have led to an understanding of the signalling pathways and the cellular feedback loops that are involved. The contribution of eosinophils to the asthmatic phenotype, which remains an area of some controversy, has been extensively dissected using ovalbumin challenge models [9, 90-93]. Also of particular interest have been studies that have identified the role of eosinophils in the afferent limb of the allergic response [87, 94, 95].

These models have been used to perform some fascinating studies of the mechanisms of asthmatic inflammation. Notably, such investigations have helped to define the role of chemokines, other chemoattractant inflammatory mediators, and adhesion molecules in the recruitment of leucocytes in allergic inflammation [96-105]. More recently, a number of studies have helped to identify novel inflammatory pathways, such as those involving arginase [106, 107] or acidic mammalian chitinase [108].

A variety of experimental studies have also contributed to an understanding of the mechanisms underlying airway wall remodelling. Thus, for example, the role of Th2 cytokines in the pathways leading to goblet cell hyperplasia/metaplasia has been demonstrated using ovalbumin challenge models [31, 109-112]. In our chronic challenge model, we defined a critical role for $CD4^+$ T cells in regulating subepithelial fibrosis [113]. Other studies have elucidated the contribution of cytokines such as IL-5 and IL-13 to subepithelial/airway fibrosis, both directly and via regulation of growth factors such as transforming growth factor- β [114-118].

Experiments in ovalbumin challenge models have helped to identify cytokines that regulate the development of AHR in asthma. It is noteworthy, however, that results obtained in chronic challenge models have sometimes been significantly at variance with those in short-term high-level challenge models, possibly reflecting the difference in the pattern of airway/parenchymal lesions. An important role for IL-13 has been identified in both acute and chronic challenge models [117-119]. Nevertheless, Th2 signalling pathways do not operate in the same way in the two types of models, because STAT6-deficient animals exhibit markedly diminished AHR in short-term challenge models [31, 120], whereas hyperresponsiveness is undiminished or even exaggerated in these mice in chronic challenge models [121]. In studies in our model of chronic asthma, we also identified a potentially

significant role for interferon- γ in the development of AHR [118, 122].

An important unanswered question in the pathogenesis of allergic asthma is the role of infections in early life in triggering or protecting from development of disease. This has been an area of considerable research interest in recent years. One strongly supported view is that infections in early life and/or repeated exposures to microbial products protect against subsequent development of allergic diseases, including asthma. Proponents of this argue that such exposures program the adaptive immune response away from the "default" Th2-dominated profile of cytokine production by CD4⁺ T cells [123] and may be critical to the development of asthma. The concept has come to be known as the hygiene hypothesis and is supported by numerous epidemiological studies [124, 125]. The underlying mechanism may involve stimulation of the immune system *via* toll-like receptors e.g. TLR-2 and TLR-4 [126] although the interactions involved are evidently complex. A contrasting viewpoint from other studies is that some early life viral infections, notably by respiratory syncytial virus (RSV), predispose to development of asthma [127, 128], especially with infection in the first 3 months of life [129]. Using ovalbumin challenge models, the crucial impact of timing of infection relative to allergic sensitisation has been demonstrated [130]. Moreover, infection by viruses such as RSV and its murine counterpart, pneumonia virus of mice (PVM), has been shown to promote allergic sensitisation, whereas influenza virus infection appears to suppress the allergic response [131]. Longitudinal studies of the interaction between early life viral infection, using PVM as the species-specific model for infection with RSV [132, 133], and allergic sensitisation, using a model of chronic ovalbumin challenge, are currently a major focus of work in our laboratories. Also of interest is the potential role of bacterial infections and/or immunisation with bacterial products as a protective or therapeutic approach, again using ovalbumin challenge models [133a].

Another challenging area of study is the interaction between viral infections and allergic asthma in the development of exacerbations of disease. Exacerbations in asthmatics are most often triggered by viral infections, especially rhinovirus [134], although allergen exposure evidently synergises with infection, eliciting a more severe inflammatory response [135, 136]. Appropriate models of rhinovirus infection in mice on a background of low-level allergen challenge would help to elucidate the pathogenesis of this important clinical interaction.

Identifying Novel Therapeutic Targets

Ovalbumin challenge models have been extensively used for pre-clinical evaluation of potential therapeutic agents for asthma. In assessing novel treatment approaches, it must be recognised that the appropriate choice of therapy in clinical asthma is determined by the duration and severity of disease. In this context, the choice of experimental model may have an important influence on the data obtained e.g. models that elicit milder inflammatory changes may be more sensitive for evaluating therapeutic effects.

Among novel anti-inflammatory agents that have been investigated in ovalbumin challenge models have been a

wide variety of extracts from medicinal plants, including traditional herbal mixtures, as well as novel defined compounds. The latter include inhibitors of phosphodiesterase-4, which have considerable promise for treatment of asthma [137-139]. Other approaches that have been the focus of recent attention include inhibitors of the synthesis of inflammatory mediators or their functional antagonists ([140, 141], inhibitors of cellular receptors critical to the induction of allergic inflammation e.g. CCR3 [142], antibodies to various cytokines [118, 143, 144] and non-antigen-specific immune response modifiers [36, 145]. Numerous more radical therapeutic options, for example involving antisense oligonucleotides and DNA immunisation, have also been investigated [146-151].

An area of current interest in our laboratories is the role of microRNAs (miRNAs), which are a recently discovered class of small non-coding gene regulatory molecules. Individual miRNA or miRNA clusters may regulate extensive transcriptional programs or the transcription of families of genes. Thus, modulation of specific miRNA by inhibiting function or by augmenting activity is emerging as a novel and specific means for broad-spectrum suppression of the inflammatory response, including in diseases such as asthma [152, 153]. Ovalbumin challenge models provide a particularly useful system in which to assess the potential of this approach.

CONCLUSION

Models of asthma based on ovalbumin challenge in mice have limitations, which need to be recognised when attempting to interpret experimental findings. Nevertheless, careful use of well-defined models to answer specific questions can contribute to understanding pathogenesis, as well as to identifying and investigating potential therapeutic targets in asthma.

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REFERENCES

- [1] Kumar, R.K. and Foster, P.S. (2002) *Am. J. Respir. Cell Mol. Biol.*, **27**, 267-272.
- [2] Gummy, A.; Louis, J.A. and Launois, P. (2004) *Int. J. Parasitol.*, **34**, 433-444.
- [3] Brewer, J.P.; Kisselgof, A.B. and Martin, T.R. (1999) *Am. J. Respir. Crit. Care Med.*, **160**, 1150-1156.
- [4] Whitehead, G.S.; Walker, J.K.; Berman, K.G.; Foster, W.M. and Schwartz, D.A. (2003) *Am. J. Physiol. Lung Cell Mol. Physiol.*, **285**, L32-42.
- [5] Herz, U.; Braun, A.; Ruckert, R. and Renz, H. (1998) *Clin. Exp. Allergy*, **28**, 625-634.
- [6] Webb, D.C.; Matthaie, K.I.; Cai, Y.; McKenzie, A.N. and Foster, P.S. (2004) *J. Immunol.*, **172**, 1092-1098.
- [7] Shinagawa, K. and Kojima, M. (2003) *Am. J. Respir. Crit. Care Med.*, **168**, 959-967.
- [8] Nakajima, H.; Iwamoto, I.; Tomoe, S.; Matsumura, R.; Tomioka, H.; Takatsu, K. and Yoshida, S. (1992) *Am. Rev. Respir. Dis.*, **146**, 374-377.
- [9] Wilder, J.A.; Collie, D.D.; Wilson, B.S.; Bice, D.E.; Lyons, C.R. and Lipscomb, M.F. (1999) *Am. J. Respir. Cell Mol. Biol.*, **20**, 1326-1334.

- [10] Sakai, K.; Yokoyama, A.; Kohno, N. and Hiwada, K. (1999) *Clin. Exp. Immunol.*, **118**, 9-15.
- [11] Trifilieff, A.; El-Hashim, A. and Bertrand, C. (2000) *Am. J. Physiol. Lung Cell Mol. Physiol.*, **279**, L1120-1128.
- [12] Hessel, E.M.; van Oosterhout, A.J.M.; Hofstra, C.L.; de Bie, J.J.; Garssen, J.; van Loveren, H.; Verheyen, A.K.C.P.; Savelkoul, H.F.J. and Nijkamp, F.J. (1995) *Eur. J. Pharmacol.*, **293**, 401-412.
- [13] Corry, D.B.; Folkesson, H.G.; Warnock, M.L.; Erle, D.J.; Matthay, M.A.; Wiener-Kronish, J.P. and Locksley, R.M. (1996) *J. Exp. Med.*, **183**, 109-117.
- [14] O'Brien, R.; Ooi, M.A.; Clarke, A.H. and Thomas, W.R. (1996) *Immunol. Cell Biol.*, **74**, 174-179.
- [15] Ichinose, T.; Takano, H.; Miyabara, Y.; Yanagisawa, R. and Sagai, M. (1997) *Toxicology*, **122**, 183-192.
- [16] Justice, J.P.; Borchers, M.T.; Lee, J.J.; Rowan, W.H.; Shibata, Y. and Van Scott, M.R. (2002) *Am. J. Physiol. Lung Cell Mol. Physiol.*, **282**, L302-309.
- [17] Hamelmann, E.; Vella, A.T.; Oshiba, A.; Kappler, J.W.; Marrack, P. and Gelfand, E.W. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 1350-1355.
- [18] Kurup, V.P.; Mauze, S.; Choi, H.; Seymour, B.W. and Coffman, R.L. (1992) *J. Immunol.*, **148**, 3783-3788.
- [19] Zhang, Y.; Lamm, W.J.E.; Albert, R.K.; Chi, E.Y.; Henderson, W.R. and Lewis, D.B. (1997) *Am. J. Respir. Crit. Care Med.*, **155**, 661-669.
- [20] Epstein, M.M. (2004) *Int. Arch. Allergy Immunol.*, **133**, 84-100.
- [21] MacLean, J.A.; Sauty, A.; Luster, A.D.; Drazen, J.M. and De Sanctis, G.T. (1999) *Am. J. Respir. Cell Mol. Biol.*, **20**, 379-387.
- [22] Blyth, D.I.; Wharton, T.F.; Pedrick, M.S.; Savage, T.J. and Sanjar, S. (2000) *Am. J. Respir. Cell Mol. Biol.*, **23**, 241-246.
- [23] Nakae, S.; Lunderius, C.; Ho, L.H.; Schafer, B.; Tsai, M. and Galli, S.J. (2007) *J. Allergy Clin. Immunol.*, **119**, 680-686.
- [24] Lloyd, C.M.; Gonzalo, J.A.; Coyle, A.J. and Gutierrez-Ramos, J.C. (2001) *Adv. Immunol.*, **77**, 263-295.
- [25] Larsen, G.L.; Renz, H.; Loader, J.E.; Bradley, K.L. and Gelfand, E.W. (1992) *J. Clin. Invest.*, **89**, 747-752.
- [26] Brusselle, G.G.; Kips, J.C.; Tavernier, J.H.; van der Heyden, J.G.; Cuvelier, C.A.; Pauwels, R.A. and Bluethmann, H. (1994) *Clin. Exp. Allergy*, **24**, 73-80.
- [27] Kung, T.T.; Jones, H.; Adams, G.K.; Umland, S.P.; Kreutner, W.; Egan, R.W.; Chapman, R.W. and Watnick, A.S. (1994) *Int. Arch. Allergy Appl. Immunol.*, **105**, 83-90.
- [28] Foster, P.S.; Hogan, S.P.; Ramsay, A.J.; Matthei, K.I. and Young, I.G. (1996) *J. Exp. Med.*, **183**, 195-201.
- [29] Blyth, D.I.; Pedrick, M.S.; Savage, T.J.; Hessel, E.M. and Fattah, D. (1996) *Am. J. Respir. Cell Mol. Biol.*, **14**, 425-438.
- [30] Hessel, E.M.; van Oosterhout, A.J.M.; van Ark, I.; van Esch, B.; Hofman, G.; van Loveren, H.; Savelkoul, H.F.J. and Nijkamp, F.P. (1997) *Am. J. Respir. Cell Mol. Biol.*, **16**, 325-334.
- [31] Kuperman, D.; Schofield, B.; Wills-Karp, M. and Grusby, M.J. (1998) *J. Exp. Med.*, **187**, 939-948.
- [32] Cieslewicz, G.; Tomkinson, A.; Adler, A.; Duez, C.; Schwarze, J.; Takeda, K.; Larson, K.A.; Lee, J.J.; Irvin, C.G. and Gelfand, E.W. (1999) *J. Clin. Invest.*, **104**, 301-308.
- [33] Temelkovski, J.; Hogan, S.P.; Shepherd, D.P.; Foster, P.S. and Kumar, R.K. (1998) *Thorax*, **53**, 849-856.
- [34] Yiamouyiannis, C.A.; Schramm, C.M.; Puddington, L.; Stengel, P.; Baradaran-Hosseini, E.; Wolyniec, W.W.; Whitely, H.E. and Thrall, R.S. (1999) *Am. J. Pathol.*, **154**, 1911-1921.
- [35] Braun, A.; Lommatzsch, M.; Mannsfeldt, A.; Neuhaus-Steinmetz, U.; Fischer, A.; Schnoy, N.; Lewin, G.R. and Renz, H. (1999) *Am. J. Respir. Cell Mol. Biol.*, **21**, 537-546.
- [36] Jain, V.V.; Kitagaki, K.; Businga, T.; Hussain, I.; George, C.; O'Shaughnessy, P. and Kline, J.N. (2002) *J. Allergy Clin. Immunol.*, **110**, 867-872.
- [37] Kenyon, N.J.; Ward, R.W. and Last, J.A. (2003) *Toxicol. Appl. Pharmacol.*, **186**, 90-100.
- [37a] Herbert, C.; Hettiaratchi, A.; Webb, D.C.; Thomas, P.S.; Foster, P.S. and Kumar, R.K. (2008) *Clin. Exp. Allergy*, (in press).
- [38] Pitchford, S.C.; Riffo-Vasquez, Y.; Sousa, A.; Momi, S.; Gresele, P.; Spina, D. and Page, C.P. (2004) *Blood*, **103**, 639-647.
- [39] McMillan, S.J. and Lloyd, C.M. (2004) *Clin. Exp. Allergy*, **34**, 497-507.
- [40] Wegmann, M.; Fehrenbach, H.; Fehrenbach, A.; Held, T.; Schramm, C.; Garn, H. and Renz, H. (2005) *Clin. Exp. Allergy*, **35**, 1263-1274.
- [41] Cohn, L. (2001) *Am. J. Respir. Cell Mol. Biol.*, **24**, 509-512.
- [42] Velan, G.M.; Kumar, R.K. and Cohen, D.D. (1993) *Pathology*, **25**, 282-290.
- [43] Kips, J.C.; Cuvelier, C.A. and Pauwels, R.A. (1992) *Am. Rev. Respir. Dis.*, **145**, 1306-1310.
- [44] Palmans, E.; Kips, J.C. and Pauwels, R.A. (2000) *Am. J. Respir. Crit. Care Med.*, **161**, 627-635.
- [45] Jungsuwadee, P.; Benkovszky, M.; Dekan, G.; Stingl, G. and Epstein, M.M. (2004) *Int. Arch. Allergy Immunol.*, **133**, 40-48.
- [46] Sakai, K.; Yokoyama, A.; Kohno, N.; Hamada, H. and Hiwada, K. (2001) *Int. Arch. Allergy Immunol.*, **126**, 126-134.
- [47] Siegle, J.S.; Hansbro, N.; Herbert, C.; Yang, M.; Foster, P.S. and Kumar, R.K. (2006) *Am. J. Respir. Cell Mol. Biol.*, **35**, 565-570.
- [48] Fulkerson, P.C.; Rothenberg, M.E. and Hogan, S.P. (2005) *Clin. Exp. Allergy*, **35**, 1251-1253.
- [49] Kumar, R.K.; Herbert, C.; Yang, M.; Koskinen, A.M.L.; McKenzie, A.N.J. and Foster, P.S. (2002) *Clin. Exp. Allergy*, **32**, 1104-1111.
- [50] Bousquet, J.; Chanez, P.; Lacoste, J.Y.; Barneon, G.; Ghavanian, N.; Enander, I.; Venge, P.; Ahlstedt, S.; Simony-Lafontaine, J.; Godard, P. and Michel, F.B. (1990) *N. Engl. J. Med.*, **323**, 1033-1039.
- [51] Korsgren, M.; Erjefalt, J.S.; Korsgren, O.; Sundler, F. and Persson, C.G.A. (1997) *J. Exp. Med.*, **185**, 885-892.
- [52] Kumar, R.K.; Thomas, P.S.; Seetoo, D.Q.; Herbert, C.; McKenzie, A.N.; Foster, P.S. and Lloyd, A.R. (2002) *Lab. Invest.*, **82**, 495-504.
- [53] Harkema, J.R.; Mariassay, A.; St George, J.; Hyde, D.M. and Plopper, C.G. In *The Airway Epithelium: Physiology, Pathophysiology and Pharmacology*, Farmer, S. G.; Hay, D. W. P., Eds. Marcel Dekker: New York, **1991**; pp 3-39.
- [54] Kumar, R.K. (2001) *Pharmacol. Ther.*, **91**, 93-104.
- [55] Ohkawara, Y.; Lei, X.F.; Stampfli, M.R.; Marshall, J.S.; Xing, Z. and Jordana, M. (1997) *Am. J. Respir. Cell Mol. Biol.*, **16**, 510-520.
- [56] Leigh, R.; Ellis, R.; Wattie, J.; Southam, D.S.; De Hoogh, M.; Gaudie, J.; O'Byrne, P.M. and Inman, M.D. (2002) *Am. J. Respir. Cell Mol. Biol.*, **27**, 526-535.
- [57] Henderson, W.R.; Tang, L.O.; Chu, S.J.; Tsao, S.M.; Chiang, G.K.; Jones, F.; Jonas, M.; Pae, C.; Wang, H. and Chi, E.Y. (2002) *Am. J. Respir. Crit. Care Med.*, **165**, 108-116.
- [58] Cho, J.Y.; Miller, M.; Baek, K.J.; Han, J.W.; Nayar, J.; Rodriguez, M.; Lee, S.Y.; McElwain, K.; McElwain, S.; Raz, E. and Broide, D.H. (2004) *Am. J. Respir. Cell Mol. Biol.*, **30**, 651-661.
- [59] Wagers, S.; Lundblad, L.K.; Ekman, M.; Irvin, C.G. and Bates, J.H. (2004) *J. Appl. Physiol.*, **96**, 2019-2027.
- [60] Wagers, S.S.; Norton, R.J.; Rinaldi, L.M.; Bates, J.H.; Sobel, B.E. and Irvin, C.G. (2004) *J. Clin. Invest.*, **114**, 104-111.
- [61] Lundblad, L.K.; Thompson-Figueroa, J.; Allen, G.B.; Rinaldi, L.; Norton, R.J.; Irvin, C.G. and Bates, J.H. (2007) *Am. J. Respir. Crit. Care Med.*, **175**, 768-774.
- [62] Jonasson, S.; Lundqvist, M.; Hedenstierna, G. and Hjoberg, J. (2007) *Am. J. Respir. Crit. Care Med.*, **175**, A156.
- [63] Raeburn, D.; Underwood, S.L. and Villamil, M.E. (1992) *J. Pharmacol. Toxicol. Methods*, **27**, 143-159.
- [64] Hessel, E.M.; Zwart, A.; Oostven, E.; Oosterhout, A.J.M.; Blyth, D.I. and Nijkamp, F.P. (1995) *J. Appl. Physiol.*, **79**, 1711-1716.
- [65] Hamelmann, E.; Schwarze, J.; Takeda, K.; Oshiba, A.; Larsen, G.L.; Irvin, C.G. and Gelfand, E.W. (1997) *Am. J. Respir. Crit. Care Med.*, **156**, 766-775.
- [66] Petak, F.; Habre, W.; Donati, Y.R.; Hantos, Z. and Barazzzone-Argiroffo, C. (2001) *J. Appl. Physiol.*, **90**, 2221-2230.
- [67] Drazen, J.M.; Finn, P.W. and De Sanctis, G.T. (1999) *Annu. Rev. Physiol.*, **61**, 593-625.
- [68] Lundblad, L.K.; Irvin, C.G.; Adler, A. and Bates, J.H. (2002) *J. Appl. Physiol.*, **93**, 1198-1207.
- [69] Adler, A.; Cieslewicz, G. and Irvin, C.G. (2004) *J. Appl. Physiol.*, **97**, 286-292.
- [70] Lomask, M. (2006) *Exp. Toxicol. Pathol.*, **57 Suppl 2**, 13-20.
- [71] Tomioka, S.; Bates, J.H. and Irvin, C.G. (2002) *J. Appl. Physiol.*, **93**, 263-270.
- [72] Bates, J.H. and Lutchen, K.R. (2005) *Respir. Physiol. Neurobiol.*, **148**, 153-164.
- [73] Glaab, T.; Ziegert, M.; Baelder, R.; Korolewicz, R.; Braun, A.; Hohlfeld, J.M.; Mitzner, W.; Krug, N. and Hoymann, H.G. (2005) *Respir. Res.*, **6**, 139.
- [74] Hoymann, H.G. (2007) *J. Pharmacol. Toxicol. Methods*, **55**, 16-26.

- [75] Weckmann, M.; Collison, A.; Simpson, J.L.; Kopp, M.V.; Wark, P.A.; Smyth, M.J.; Yagita, H.; Matthaei, K.I.; Hansbro, N.; Whitehead, B.; Gibson, P.G.; Foster, P.S. and Mattes, J. (2007) *Nat. Med.*, **13**, 1308-1315.
- [76] Irvin, C.G. and Bates, J.H. (2003) *Respir. Res.*, **4**, 4.
- [77] Persson, C.G. (2002) *Am. J. Respir. Crit. Care Med.*, **166**, 6-7.
- [78] Wenzel, S. and Holgate, S.T. (2006) *Am. J. Respir. Crit. Care Med.*, **174**, 1173-1176; discussion 1176-1178.
- [79] Kips, J.C.; Anderson, G.P.; Fredberg, J.J.; Herz, U.; Inman, M.D.; Jordana, M.; Kemeny, D.M.; Lotvall, J.; Pauwels, R.A.; Plopper, C.G.; Schmidt, D.; Sterk, P.J.; Van Oosterhout, A.J.; Vargaftig, B.B. and Chung, K.F. (2003) *Eur. Respir. J.*, **22**, 374-382.
- [80] Garlisi, C.G.; Falcone, A.; Kung, T.T.; Stelts, D.; Pennline, K.J.; Beavis, A.J.; Smith, S.R.; Egan, R.W. and Umland, S.P. (1995) *Clin. Immunol. Immunopathol.*, **75**, 75-83.
- [81] Kaminuma, O.; Mori, A.; Ogawa, K.; Nakata, A.; Kikkawa, H.; Naito, K.; Suko, M. and Okudaira, H. (1997) *Am. J. Respir. Cell Mol. Biol.*, **16**, 448-454.
- [82] Hogan, S.P.; Koskinen, A.; Matthaei, K.I.; Young, I.G. and Foster, P.S. (1998) *Am. J. Respir. Crit. Care Med.*, **157**, 210-218.
- [83] Nagai, H.; Yamaguchi, S.; Inagaki, N.; Tsuruoka, N.; Hitoshi, Y. and Takatsu, K. (1993) *Life Sci.*, **53**, PL243-PL247.
- [84] Wills-Karp, M.; Luyimbazi, J.; Xu, X.; Schofield, B.; Neben, T.Y.; Karp, C.L. and Donaldson, D.D. (1998) *Science*, **282**, 2258-2261.
- [85] Grunig, G.; Warnock, M.; Wakil, A.E.; Venkayya, R.; Brombacher, F.; Rennick, D.M.; Sheppard, D.; Mohrs, M.; Donaldson, D.D.; Locksley, R.M. and Corry, D.M. (1998) *Science*, **282**, 2261-2263.
- [86] Mattes, J.; Yang, M.; Siqueira, A.; Clark, K.; MacKenzie, J.; McKenzie, A.N.J.; Webb, D.C.; Matthaei, K.I. and Foster, P.S. (2001) *J. Immunol.*, **167**, 1683-1692.
- [87] Mattes, J.; Yang, M.; Mahalingam, S.; Kuehr, J.; Webb, D.C.; Simson, L.; Hogan, S.P.; Koskinen, A.; McKenzie, A.N.J.; Dent, L.A.; Rothenberg, M.; Matthaei, K.I.; Young, I.G. and Foster, P.S. (2002) *J. Exp. Med.*, **195**, 1433-1444.
- [88] Webb, D.C.; Mahalingam, S.; Cai, Y.; Matthaei, K.I.; Donaldson, D.D. and Foster, P.S. (2003) *Eur. J. Immunol.*, **33**, 3377-3385.
- [89] Webb, D.C.; Cai, Y.; Matthaei, K.I. and Foster, P.S. (2007) *J. Immunol.*, **178**, 219-227.
- [90] Foster, P.S.; Hogan, S.P.; Yang, M.; Mattes, J.; Young, I.G.; Matthaei, K.I.; Kumar, R.K.; Mahalingam, S. and Webb, D.C. (2002) *Trends Mol. Med.*, **8**, 162-167.
- [91] Shen, H.H.; Ochkur, S.I.; McGarry, M.P.; Crosby, J.R.; Hines, E.M.; Borchers, M.T.; Wang, H.; Biechelle, T.L.; O'Neill, K.R.; Ansary, T.L.; Colbert, D.C.; Cormier, S.A.; Justice, J.P.; Lee, N.A. and Lee, J.J. (2003) *J. Immunol.*, **170**, 3296-3305.
- [92] Humbles, A.A.; Lloyd, C.M.; McMillan, S.J.; Friend, D.S.; Xanthou, G.; McKenna, E.E.; Ghiran, S.; Gerard, N.P.; Yu, C.; Orkin, S.H. and Gerard, C. (2004) *Science*, **305**, 1776-1779.
- [93] Lee, J.J.; Dimina, D.; Macias, M.P.; Ochkur, S.I.; McGarry, M.P.; O'Neill, K.R.; Protheroe, C.; Pero, R.; Nguyen, T.; Cormier, S.A.; Lenkiewicz, E.; Colbert, D.; Rinaldi, L.; Ackerman, S.J.; Irvin, C.G. and Lee, N.A. (2004) *Science*, **305**, 1773-1776.
- [94] Shi, H.Z.; Humbles, A.; Gerard, C.; Jin, Z. and Weller, P.F. (2000) *J. Clin. Invest.*, **105**, 945-953.
- [95] MacKenzie, J.R.; Mattes, J.; Dent, L.A. and Foster, P.S. (2001) *J. Immunol.*, **167**, 3146-3155.
- [96] Nakajima, H.; Sano, H.; Nishimura, T.; Yoshida, S. and Iwamoto, I. (1994) *J. Exp. Med.*, **179**, 1145-1154.
- [97] MacLean, J.A.; Ownbey, R. and Luster, A.D. (1996) *J. Exp. Med.*, **184**, 1461-1469.
- [98] Gonzalo, J.A.; Lloyd, D.M.; Kremer, L.; Finger, E.; Martinez-A, C.; Siegelman, M.H.; Cybulsky, M. and Gutierrez-Ramos, J.C. (1996) *J. Clin. Invest.*, **98**, 2332-2345.
- [99] Henderson, W.R.; Lewis, D.B.; Albert, R.K.; Zhang, Y.; Lamm, W.J.; Chiang, G.K.; Jones, F.; Eriksen, P.; Tien, Y.T.; Jonas, M. and Chi, E.Y. (1996) *J. Exp. Med.*, **184**, 1483-1494.
- [100] Mould, A.W.; Matthei, K.I.; Young, I.G. and Foster, P.S. (1997) *J. Clin. Invest.*, **99**, 1064-1071.
- [101] Gonzalo, J.A.; Lloyd, C.M.; Wen, D.; Albar, J.P.; Wells, T.N.; Proudfoot, A.; Martinez-A, C.; Dorf, M.; Bjerke, T.; Coyle, A.J. and Gutierrez-Ramos, J.C. (1998) *J. Exp. Med.*, **188**, 157-167.
- [102] Campbell, E.M.; Kunkel, S.L.; Strieter, R.M. and Lukacs, N.W. (1998) *J. Immunol.*, **161**, 7047-7053.
- [103] Wolyniec, W.W.; De Sanctis, G.T.; Nabozny, G.; Torcellini, C.; Haynes, N.; Joetham, A.; Gelfand, E.W.; Drazen, J.M. and Noonan, T.C. (1998) *Am. J. Respir. Cell Mol. Biol.*, **18**, 777-785.
- [104] Hisada, T.; Salmon, M.; Nasuhara, Y. and Chung, K.F. (1999) *Am. J. Respir. Crit. Care Med.*, **160**, 571-575.
- [105] Hisada, T.; Hellewell, P.G.; Teixeira, M.M.; Malm, M.G.; Salmon, M.; Huang, T.J. and Chung, K.F. (1999) *Am. J. Respir. Cell Mol. Biol.*, **20**, 992-1000.
- [106] Zimmermann, N.; King, N.E.; Laporte, J.; Yang, M.; Mishra, A.; Pope, S.M.; Muntel, E.E.; Witte, D.P.; Pegg, A.A.; Foster, P.S.; Hamid, Q. and Rothenberg, M.E. (2003) *J. Clin. Invest.*, **111**, 1863-1874.
- [107] Yang, M.; Rangasamy, D.; Matthaei, K.I.; Frew, A.J.; Zimmermann, N.; Mahalingam, S.; Webb, D.C.; Tremethick, D.J.; Thompson, P.J.; Hogan, S.P.; Rothenberg, M.E.; Cowden, W.B. and Foster, P.S. (2006) *J. Immunol.*, **177**, 5595-5603.
- [108] Zhu, Z.; Zheng, T.; Homer, R.J.; Kim, Y.K.; Chen, N.Y.; Cohn, L.; Hamid, Q. and Elias, J.A. (2004) *Science*, **304**, 1678-1682.
- [109] Cohn, L.; Homer, R.J.; Marinov, A.; Rankin, J. and Bottomly, K. (1997) *J. Exp. Med.*, **186**, 1737-1747.
- [110] Townsend, M.J.; Fallon, P.G.; Matthews, D.J.; Smith, P.; Jolin, H.E. and McKenzie, A.N.J. (2000) *Immunity*, **13**, 573-583.
- [111] Shim, J.J.; Dabbagh, K.; Ueki, I.F.; Dao-Pick, T.; Burgel, P.R.; Takeyama, K.; Tam, D.C. and Nadel, J.A. (2001) *Am. J. Physiol.*, **280**, L134-L140.
- [112] Whittaker, L.; Niu, N.; Temann, U.A.; Stoddard, A.; Flavell, R.A.; Ray, A.; Homer, R.J. and Cohn, L. (2002) *Am. J. Respir. Cell Mol. Biol.*, **27**, 593-602.
- [113] Foster, P.S.; Yang, M.; Herbert, C. and Kumar, R.K. (2002) *Lab. Invest.*, **82**, 455-462.
- [114] Tanaka, H.; Komai, M.; Nagao, K.; Ishizaki, M.; Kajiwar, D.; Takatsu, K.; Delespesse, G. and Nagai, H. (2004) *Am. J. Respir. Cell Mol. Biol.*, **31**, 62-68.
- [115] Cho, J.Y.; Miller, M.; Baek, K.J.; Han, J.W.; Nayar, J.; Lee, S.Y.; McElwain, K.; McElwain, S.; Friedman, S. and Broide, D.H. (2004) *J. Clin. Invest.*, **113**, 551-560.
- [116] Kumar, R.K.; Herbert, C. and Foster, P.S. (2004) *Clin. Exp. Allergy*, **34**, 567-575.
- [117] Yang, G.; Volk, A.; Petley, T.; Emmell, E.; Giles-Komar, J.; Shang, X.; Li, J.; Das, A.M.; Shealy, D.; Griswold, D.E. and Li, L. (2004) *Cytokine*, **28**, 224-232.
- [118] Kumar, R.K.; Herbert, C.; Webb, D.C.; Li, L. and Foster, P.S. (2004) *Am. J. Respir. Crit. Care Med.*, **170**, 1043-1048.
- [119] Walter, D.M.; McIntire, J.J.; Berry, G.; McKenzie, A.N.; Donaldson, D.D.; DeKruyff, R.H. and Umetsu, D.T. (2001) *J. Immunol.*, **167**, 4668-4675.
- [120] Akimoto, T.; Numata, F.; Tamura, M.; Takata, Y.; Higashida, N.; Takashi, T.; Takeda, K. and Akira, S. (1998) *J. Exp. Med.*, **187**, 1537-1542.
- [121] Foster, P.S.; Webb, D.C.; Yang, M.; Herbert, C. and Kumar, R.K. (2003) *Clin. Exp. Allergy*, **33**, 688-695.
- [122] Kumar, R.K.; Webb, D.C.; Herbert, C. and Foster, P.S. (2006) *Inflamm. Allergy Drug Targets*, **5**, 253-256.
- [123] Rowe, J.; Macaubas, C.; Monger, T.M.; Holt, B.J.; Harvey, J.; Poolman, J.T.; Sly, P.D. and Holt, P.G. (2000) *Infect. Immun.*, **68**, 3873-3877.
- [124] Weiss, S.T. (2002) *N. Engl. J. Med.*, **347**, 930-931.
- [125] Kamradt, T.; Goggel, R. and Erb, K.J. (2005) *Trends Immunol.*, **26**, 260-267.
- [126] Velasco, G.; Campo, M.; Manrique, O.J.; Bellou, A.; He, H.; Ares-tides, R.S.; Schaub, B.; Perkins, D.L. and Finn, P.W. (2005) *Am. J. Respir. Cell Mol. Biol.*, **32**, 218-224.
- [127] Lemanske, R.F. (2004) *J. Allergy Clin. Immunol.*, **114**, 1023-1026.
- [128] Sigurs, N.; Gustafsson, P.M.; Bjarnason, R.; Lundberg, F.; Schmidt, S.; Sigurbergsson, F. and Kjellman, B. (2005) *Am. J. Respir. Crit. Care Med.*, **171**, 137-141.
- [129] Kristjansson, S.; Bjarnason, S.P.; Wennergren, G.; Palsdottir, A.H.; Arnadottir, T.; Haraldsson, A. and Jonsdottir, I. (2005) *J. Allergy Clin. Immunol.*, **116**, 805-811.
- [130] Peebles, R.S., Jr.; Hashimoto, K.; Collins, R.D.; Jarzecka, K.; Furlong, J.; Mitchell, D.B.; Sheller, J.R. and Graham, B.S. (2001) *J. Infect. Dis.*, **184**, 1374-1379.
- [131] Barends, M.; de Rond, L.G.; Dormans, J.; van Oosten, M.; Boelen, A.; Neijens, H.J.; Osterhaus, A.D. and Kimman, T.G. (2004) *Clin. Exp. Allergy*, **34**, 488-496.
- [132] Domachowske, J.B.; Bonville, C.A. and Rosenberg, H.F. (2004) *Pediatr. Infect. Dis. J.*, **23**, S228-S234.
- [133] Rosenberg, H.F.; Bonville, C.A.; Easton, A.J. and Domachowske, J.B. (2005) *Pharmacol. Ther.*, **105**, 1-6.

- [133a] Preston, J.A.; Essilfie, A.T.; Horvat, J.C.; Wade, M.A.; Beagley, K.W.; Gibson, P.G.; Foster, P.S. and Hansbro, P.M. (2007) *Vaccine*, **25**, 8154-8162.
- [134] Grissell, T.V.; Powell, H.; Shafren, D.R.; Boyle, M.J.; Hensley, M.J.; Jones, P.D.; Whitehead, B.F. and Gibson, P.G. (2005) *Am. J. Respir. Crit. Care Med.*, **172**, 433-439.
- [135] Green, R.M.; Custovic, A.; Sanderson, G.; Hunter, J.; Johnston, S.L. and Woodcock, A. (2002) *BMJ*, **324**, 763.
- [136] Proud, D. and Chow, C.W. (2006) *Am. J. Respir. Cell Mol. Biol.*, **35**, 513-518.
- [137] Kanehiro, A.; Ikemura, T.; Makela, M.J.; Lahn, M.; Joetham, A.; Dakhama, A. and Gelfand, E.W. (2001) *Am. J. Respir. Crit. Care Med.*, **163**, 173-184.
- [138] Kumar, R.K.; Herbert, C.; Thomas, P.S.; Wollin, L.; Beume, R.; Yang, M.; Webb, D.C. and Foster, P.S. (2003) *J. Pharmacol. Exp. Ther.*, **307**, 349-355.
- [139] Deng, Y.M.; Xie, Q.M.; Tang, H.F.; Sun, J.G.; Deng, J.F.; Chen, J.Q. and Yang, S.Y. (2006) *Eur. J. Pharmacol.*, **547**, 125-135.
- [140] Oh, S.W.; Pae, C.I.; Lee, D.K.; Jones, F.; Chiang, G.K.; Kim, H.O.; Moon, S.H.; Cao, B.; Ogbu, C.; Jeong, K.W.; Kozu, G.; Nakanishi, H.; Kahn, M.; Chi, E.Y. and Henderson, W.R. (2002) *J. Immunol.*, **168**, 1992-2000.
- [141] Henderson, W.R., Jr.; Chiang, G.K.; Tien, Y.T. and Chi, E.Y. (2006) *Am. J. Respir. Crit. Care Med.*, **173**, 718-728.
- [142] Wegmann, M.; Goggel, R.; Sel, S.; Erb, K.J.; Kalkbrenner, F.; Renz, H. and Garn, H. (2007) *Am. J. Respir. Cell Mol. Biol.*, **36**, 61-67.
- [143] Ma, Y.; Hayglass, K.T.; Becker, A.B.; Fan, Y.; Yang, X.; Basu, S.; Srinivasan, G.; Simons, F.E.; Halayko, A.J. and Peng, Z. (2007) *Am. J. Respir. Crit. Care Med.*, **176**, 439-445.
- [144] Ma, Y.; Hayglass, K.T.; Becker, A.B.; Halayko, A.J.; Basu, S.; Simons, F.E. and Peng, Z. (2007) *Allergy*, **62**, 675-682.
- [145] Quarcoo, D.; Weixler, S.; Joachim, R.A.; Stock, P.; Kallinich, T.; Ahrens, B. and Hamelmann, E. (2004) *Clin. Exp. Allergy*, **34**, 1314-1320.
- [146] Finotto, S.; De Sanctis, G.T.; Lehr, H.A.; Herz, U.; Buerke, M.; Schipp, M.; Bartsch, B.; Atreya, R.; Schmitt, E.; Galle, P.R.; Renz, H. and Neurath, M.F. (2001) *J. Exp. Med.*, **193**, 1247-1260.
- [147] Hertz, M.; Mahalingam, S.; Dalum, I.; Klysner, S.; Mattes, J.; Neisig, A.; Mouritsen, S.; Foster, P.S. and Gautam, A. (2001) *J. Immunol.*, **167**, 3792-3799.
- [148] Zuany-Amorim, C.; Manlius, C.; Dalum, I.; Jensen, M.R.; Gautam, A.; Pay, G.; Mouritsen, S. and Walker, C. (2004) *Int. Arch. Allergy Immunol.*, **133**, 154-163.
- [149] Duan, W.; Chan, J.H.; McKay, K.; Crosby, J.R.; Choo, H.H.; Leung, B.P.; Karras, J.G. and Wong, W.S. (2005) *Am. J. Respir. Crit. Care Med.*, **171**, 571-578.
- [150] Crosby, J.R.; Guha, M.; Tung, D.; Miller, D.A.; Bender, B.; Condon, T.P.; York-DeFalco, C.; Geary, R.S.; Monia, B.P.; Karras, J.G. and Gregory, S.A. (2007) *J. Pharmacol. Exp. Ther.*, **321**, 938-946.
- [151] Karras, J.G.; Crosby, J.R.; Guha, M.; Tung, D.; Miller, D.A.; Gaarde, W.A.; Geary, R.S.; Monia, B.P. and Gregory, S.A. (2007) *Am. J. Respir. Cell Mol. Biol.*, **36**, 276-285.
- [152] Yang, M.; Mattes, J.; Hansbro, P. and Foster, P.S. (2006) *Drug Discov. Today*, **3**, 325-332.
- [153] Mattes, J.; Yang, M. and Foster, P.S. (2007) *Am. J. Respir. Cell Mol. Biol.*, **36**, 8-12.