

Improved Mouse Models of Allergy and Allergic Asthma - Chances Beyond Ovalbumin

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Abstract: Allergic asthma is defined as a hypersensitivity reaction of the lung towards *per se* harmless antigens, e.g. pollen and house dust mite, accompanied with a chronic eosinophilic inflammation of the lung. During the course of the disease, physiological and structural changes in the lung occur, i.e. airway hyperresponsiveness, restricted airflow and airway remodelling. In addition to ovalbumin-induced mouse models of acute asthma, recently new models were developed, which show a closer resemblance to human asthma, both regarding the induction of characteristics of chronic allergic inflammation and the use of clinical relevant allergens. Moreover, attention is paid on the influence of adjuvants or the route of sensitisation on the protocol outcomes. The effort spent in development of these new models will be worthwhile, especially for research in the field of immuno-therapy. These improved animal models may broaden the knowledge of the disease and thereby provide new strategies for preventive and therapeutic interventions.

Key Words: Mouse models, allergic asthma, chronic inflammation, clinical relevant allergens.

INTRODUCTION

When discussing appropriate mouse models of allergic asthma, two main points have to be taken into consideration. Firstly, symptoms of allergic asthma have to be reflected. Patients with allergic asthma characteristically suffer from recurrent attacks of breathlessness and wheezing, caused by an airway hyper-reactivity towards inhaled allergens and unspecific stimuli as cold air or cigarette smoke. Furthermore, a chronic eosinophilic inflammation of the airways is prominent. Secondly, although animal models are *per se* restricted to what they are- models, the investigator should exert him- or herself to resemble the human pathogenesis as closely as possible. The general aim of these efforts is to reflect human pathology and thereby allow relevant research on patho-mechanisms and reliable predictions of the therapeutic value new intervention strategies may bear.

Mouse models were and are a basic tool in asthma research and provided us with the opportunity to investigate the establishment of allergic asthma. This led to valuable insight in the principal mechanisms and mediators of the disease. However, some therapies promising in standard mouse models turned out to be less effective when transferred to patients, pointing to a restricted comparability. For example, the role of interleukin (IL)-5 is controversially discussed: at least in mice, IL-5 plays a central role in asthma [1]. In patients, therapies directed against IL-5 actually reduced allergic inflammation, however, airway hyper-responsiveness was, if at all, only moderately ameliorated [2-3]. To overcome these limitations in prediction, a lot of effort was made to improve the standard ovalbumin (OVA)-models, both

in substituting acute by chronic models and in replacing the model allergen OVA by clinical relevant allergens.

This review provides an overview of the improvements mouse models of asthma achieved in the last years, and furthermore, briefly introduces a selection of non-OVA mouse models currently used. These new models differ in the inbred mouse strain applied, in the model allergens, application of an adjuvant, and the duration. In these new and optimised models very distinct question can be investigated, for example from the field of immunotherapy.

One Step Closer: Modelling Chronic Allergic Asthma

Although one can hardly name “the” standard mouse model of OVA-induced allergic asthma, research performed in the one or the other type of acute-phase OVA-models supposedly delivered the main part of nowadays knowledge regarding allergic asthma. By studying acute models, the contribution of a variety of cells and mediators to the clinical picture of allergic asthma could be described.

However, while applying acute OVA models fundamental differences to the clinic became more and more prominent, emphasising that allergic asthma is in fact a chronic disease. In addition, the role of different cell types and factors involved in acute asthma might be altered during chronicisation and have to be clarified. Therefore, the need for new asthma models emerged. A variety of mouse models came up recently to study mechanisms leading to chronic allergic asthma. These protocols aim to induce features of chronic allergic asthma in experimental animals which could not be found in adequate amount in so called acute models, as there is perpetuated eosinophilic inflammation and structural remodelling of the lung.

Most commonly these models use OVA and adjuvant, e.g. alum, for sensitisation to induce allergen-specific Th2

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driven immune responses (reviewed in detail by Kumar *et al.* in this issue). To provoke a chronic asthmatic phenotype in the lungs, mice are repeatedly challenged with allergen during a weeks' period up to several months [4-9]. Outcomes of these protocols demonstrate that the known main effector cells of acute allergic responses also contribute to the chronic phase, as there are CD4⁺ lymphocytes [10], eosinophils [reviewed in 11] and mast cells. As a finding absent in acute models, increasing numbers of mast cells could be found in chronically challenged mice [8]. Mast cells contribute, but are not fully indispensable, to changes related to the chronic status [12].

Moreover, these models show the ability to reflect airway remodelling including goblet-cell hyperplasia [12-13], epithelial shedding, mucus hypersecretion [14], subepithelial fibrosis [15], smooth muscle hypertrophy [13] and changes in the extracellular matrix (ECM). Direct and indirect contribution of cytokines (IL-13, IL-10 and especially transforming growth-factor (TGF)- β) and endopeptidases (matrix metalloproteinases and tissue inhibitors of metalloproteinases [16-18]) to these remodelling processes could be demonstrated [13, 19-25]. These fibrotic processes are thought to result or at least contribute to the reduction of lung function [26].

Additionally, physiological parameters can be induced and determined in mice, which reflect an airway hyperresponsiveness (AHR) to unspecific stimuli [6, 12, 27]. Hereby, continued allergen challenges seem to be necessary: Studies showed an (alleviated) perpetuated airway inflammation, while AHR was lost after cessation of challenges [13, 28]. However, AHR was absent in animals chronically challenged with low-level allergen doses as well, but could rapidly be induced when a single moderate dose was administered [9]. Interestingly, during the chronic phase elevated levels of a classically T helper (h)1-related cytokine, namely interferon- γ , can be found [13], which may contribute to chronic AHR [29].

Due to their closer resemblance, chronic models have clear advantages in pharmacological studies. This is demonstrated by the attenuating effects on chronic inflammation by cytosolic phospholipase A2 inhibitors, an effect which could not be observed during the acute status [30]. Further, the beneficial effects of the cysteinyl leukotriene-receptor blocking compound montelukast were demonstrated in a chronic model. Here, a decrease in inflammation and fibrosis, mucus plugging, smooth muscle hyperplasia and reduction of IL-4 and IL-13 mRNA in lung and bronchoalveolar lavage fluid (BALF) protein was achieved [31].

Limitations of Chronic Ovalbumin-Asthma Models

Although these chronic models represent a clear improvement of acute-phase models, they have to face some practical limitations.

One basic problem is the resolution of airway eosinophilia in long-time protocols [28-29]. This resolution occurs despite the fact that eosinophil survival prolonging mediators are increasing throughout the duration of the protocols: As an example, Th2 cytokines, as well as IL-1 β and tumor necrosis factor (TNF)- α [32] up-regulate the synthesis of neu-

rotrophins, which protect eosinophils of asthmatic patients from apoptosis [33]. An alleviation of eosinophilic inflammation is frequently observed after continued OVA-challenges, but appears to be disconnected to lipopolysaccharide (LPS)-contaminations of the OVA-preparations used [34]: thus, no modulation of the immune response by this potent toll-like receptor agonist could be identified as a process signing responsible.

Usually, long-time OVA-challenge models are referred to as tolerance models, pointing to another limitation: the induction of tolerance and immuno-suppression [35]. Although tolerance is a naturally occurring and beneficial process in allergic diseases, the question remains what causes the immune system of allergic patients to be unable to cope with the disease. The fact that tolerance can be induced easily in mice complicates to set up a model that replicates human pathology.

Animal Models of Allergy and Allergic Asthma Induced by Clinically Relevant Allergens

To overcome the limitations OVA-induced mouse models show, investigators chose different starting points: First, OVA is replaced by clinical relevant allergens. Second, models using relevant allergens are prolonged, to resemble chronic allergic inflammation and chronic asthma. Third, attention is paid on the influence of genetics and adjuvants on the protocol outcome.

In the following, examples for these three approaches will be given. In the second part of this review, special attention will be turned on grass-pollen allergens, which will serve as an example to clarify the considerations and potential obstacles these new approaches may contain.

Clinical Relevant Allergens

Several groups worked on new models which use human relevant allergens either as recombinant proteins or extract preparations for induction of allergic inflammation and asthma in mice.

Important structural findings distinguish these allergens from OVA: with 45 kDa, OVA is relative big in size, and a monovalent protein [36]. Many clinical relevant allergens are indeed multivalent [37], or exclusively recognised by membrane-bound immuno globuline (IgE) when they occur in a dimeric structure. This was shown for the birch major allergen Bet v1, which is silent in its 17 kDa form but induces IgE-mediated degranulation when identical epitopes of a 34 kDa dimer can be bound [38].

Moreover, the induced immune responses are altered, e.g. a typically IgE-dominated anti-OVA response is partly replaced by IgG1-antibodies in the chronic phase [39].

As in acute-phase models, the detailed protocols are highly diverse. A variety of allergens as well as different adjuvants are combined, as listed exemplarily in Table 1.

Interestingly, the allergenicity of different preparations seems to vary: The sensitising potential of purified natural major allergen is stronger than that of recombinant allergens, as shown for IgE-induction in olive pollen allergen models [43].

Table 1. Alternative Allergens in Mouse Models of Allergy and Acute-Phase Allergic Asthma

Allergen	Mouse Strain	Adjuvant	Boost	Challenge	Protocol Outcome	Ref.
Cockroach, rBla g2 House dust mite, rDer f1	A/J	Alum, i.p.	Alum, i.p., d 7	d 14, oro-tracheally	Allergic inflammation, AHR (rBla g2)	[40]
House dust mite-extract	BALB/c	HDM/CFA, s.c. 2x PT i.p.	HDM/IFA, d 7	6x/week, 6 weeks, followed by 2x/week, 3 weeks, followed by 3x on consecutive days, all i.n.	Allergic inflammation, AHR	[41]
House dust mite-extract	C57BL/6	-, s.c.d 1	-, i.p. d 3, 6, 8, 10, 13, 15, 17	i.t., d 32, 34, 37, 79, 81	Allergic inflammation, IgE, specific IgG1	[42]
Olive pollen: natural and recombinant Ole e1	BALB/c	Alum, d 0	d 7, 14, 21	-	IgE, specific IgE, allergen-induced proliferation of splenocytes	[43]
Aspergillus fumigatus-extract	BALB/c C57BL/6	IFA, i.p and s.c.	-, d 14, inhalative	d 21, inhalative	AHR, Allergic inflammation, IgE, Goblet-cell metaplasia	[44-45]
Timothy grass pollen (rPhl p5)	BALB/c	Alum, s.c.	d 21	-	Specific IgE, IgA, IgG1, IgM, basophil-degranulation	[46]

CFA= complete Freud's adjuvant, IFA= incomplete Freud's adjuvant, PT= pertussis toxin

Many investigators choose allergen extracts instead of purified major allergens. This may mean a loss of control over the distinct proteins for sensitisation, because an extract summarises a variety of different allergens. However, it is in fact this mixture of allergens a patient would be exposed to simultaneously.

Application of New Models to Allergen-Specific Questions

To claim that these models were developed only to circumvent problems one has to face when working with OVA-models would definitively suppress a wide area of research, in which they are tremendously important and necessary. In these new and optimised animal models very distinct questions can exclusively be investigated, for example from the field of desensitisation or specific immunotherapy.

As therapeutics in specific immunotherapy, recombinant allergens are frequently used and of high value. Here, the main clinical allergens are investigated, e.g. of birch (rBet v1 [47], olive tree (rOle e1 [43]) and Timothy grass (rPhl p5 [48]). These major allergens are applied as recombinant proteins or peptides, as their dominant T-cell epitope, as DNA-vaccines or, as a more "classical" approach, as antibodies against the major allergen. Some examples are given in Table 2.

Relevant Allergens in Chronic Models of Allergy and Asthma

If so much attention is paid to improve the resemblance of the experimental model to human pathology, one could not ignore the differences between acute- and chronic-phase models as described earlier. Therefore, chronic models using

Table 2. Alternative Mouse Models of Allergy and Acute-Phase Allergic Asthma in the Field of Immunotherapy

Allergen	Mouse Strain	Adjuvant	Boost/ Challenge	Treatment	Treatment Outcome	Ref.
Birch pollen (rBet v1)	BALB/c	Alum	Four times, 12 d apart i.p.	Dominant T-cell epitope (Bet v1-aa 139-152) s.c, both before and after sensitisation	reduced T-cell proliferation and skin-prick test reactivity	[49]
Rye grass pollen (Lol p 1)	BALB/c	Alum		Anti-Lol p1 monoclonal antibody	Suppression of specific IgE/IgG1-production	[50]
Timothy grass pollen (rPhl p5)	BALB/c	Alum, d 42 s.c.	d 58, 72 s.c. /rPhl p. d 101 i.n.	Replicon-based DNA vaccine intradermal, d 0, 7, 21	Th1-shift, reduction of allergic inflammation and histopathology, alleviated AHR	[51]
Poly-sensitisation to timothy grass and birch pollen Bet v1 Phl p1 Phl p5	BALB/c	Alum	d 26, 36, 50 / birch and grass pollen extract as aerosol d 28, 29	d 0, 7, 14, recombinant peptide for each allergen or hybrid peptide	Reduced airway inflammation, reduced IgE-mediated basophil-degranulation	[52]

Table 3. Alternative Allergens in Mouse Models of Chronic Allergic Asthma

Allergen source	Mouse Strain	Adjuvant and Route of Sensitisation	Boost	Challenge	Outcome	Ref.
Olive pollen extract	BALB/c	Alum, s.c.	1x/week 3 weeks	Week 10, 2x extract i.n.	Specific IgE/IgG1, AHR, Th2- cytokine- profile in splenocyte recall	[59]
Alternaria alternata-extract	BALB/c	-	-	Mondays and Fridays, 10-12	Specific IgE/IgG1, Inflammation, AHR, Remodelling	[57]
Cladosporium herbarum-spores	BALB/c	-	-	weeks i.n.		
Aspergillus fumigatus	CBA/J	IFA, i.p. and s.c.-extract	3x extract i.n.	1x conidia i.t.	IgE, Remodelling, AHR, Allergic inflammation, Th2- cytokine- profile	[60]
Dermatophagoides pteronyssinus-extract	BALB/c	-	-	5 consecutive days/week, 5 weeks i.n.	Specific IgG1, Th2- cytokine- profile in splenocyte recall, Allergic inflammation, Remodelling, AHR	[58]

relevant allergens are an important innovation (for examples, see Table 3).

However, still two basic considerations have to be taken into account: the use of an adjuvant, as well the route of sensitisation.

By the use of adjuvants a rapid and intense immune response is induced, shortening the time of sensitisation and leading to severely inflamed tissues. Intraperitoneal sensitisations with an adjuvant might on the one hand amplify and quicken the sensitisation, but remain on the other hand not comparable to the human situation at all. In some models, the use of an adjuvant can influence the investigation importantly [53]. Seitzer *et al.* [54] demonstrated in their i.p.-model of *Phleum pratense*-extract sensitisation a clear induction of unspecific IgE by the administration of alum, exceeding the IgE-induction by the extract without additional adjuvant.

Also when relevant allergens are applied as therapeutics, the immuno-modulatory effects of adjuvants are dependent on the allergen under investigations, or used in the model. For example, cholera-toxin coupled to Bet v1 acted adjuvant, whereas it showed a tolerogenic capacity in an OVA-model [47].

But not only does the use of (and if, which) adjuvant influence the protocol outcome. Although it is still not clarified *via* which route patients are primarily sensitised to allergens, intra-peritoneal injections evidently remain artificial. Currently little is known about differences in the immunologic reaction when the provocation route is altered, although there are some evidences from animal models that the pace of sensitisation is changed (topic vs. respiratory sensitisation in BN rats, reviewed in [55]).

To reach a further step closer to human pathology, not only the allergens used in a murine model of allergic airway inflammation should be relevant for human pathogenesis, but also the route of sensitisation should at least be likely, or possible. Moreover, a milder course of the disease might

reflect the human pathology more closely. Recently, the first acute and chronic models were described in which human relevant allergens could induce allergy symptoms by intranasal administration [56-58].

THE WAY TO A CHRONIC MODEL OF GRASS-POLLEN ALLERGEN-INDUCED ALLERGY: CONSIDERATIONS AND OBSTACLES

Grass-Pollen as Allergen Carrier

Although pollen grains are in fact too big to be inhaled to the deeper airways and cause allergic asthma at this site, they remain a main source for human relevant allergens. In fact, different factors cause a rupture of the pollen grain and its fragmentation into small, respirable particles, which was demonstrated for pollen of birch as well as for timothy grass [61-62]. Among these factors humidity and wind shaking of the anthers play an important role. The small fragments carry the allergen in question, for example, timothy grass major allergen 5 (Phl p5).

The major allergen 5 of Timothy grass is an RNase involved in the plant's defence against infections [63]. It is believed to be locally associated with pollen starch granules (PSGs), but also in the cytosol of pollen grains [64-65]. In timothy grass, the major allergen groups 1 and 5 are estimated to represent the most important allergen groups, whereby "almost 90% of grass pollen-allergic patients are sensitized against group 5 grass pollen allergens" [48]. These allergens show high immunogenicity both in mice and in humans [39, 66]. Meanwhile recombinant Phl p5 as well as highly standardised allergen extract preparations can be purchased from commercial distributors. This allows the establishment of reliable and robust experimental protocols.

Variations in Natural Allergen Sources-"Natural" Endogenous Adjuvants

Pollen grains are a main source for seasonal allergens, causing allergic disorders as rhinitis, conjunctivitis and

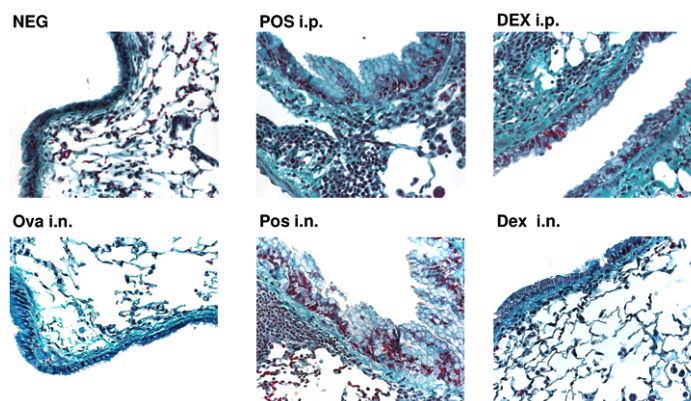


Fig. (1). Lung sections. 24 h after the 22nd grass-pollen allergen provocation, lungs were perfused via the left ventricle and a 4% formaldehyde solution was instilled. Lungs were fixated over night in 4% formaldehyde, bedded in paraffin and cut into 1 μ m slices. Masson Goldner Trichrome stain was performed. Photos were taken at 400x magnification from the distal part of the main bronchus. Upper row: i.p. sensitised groups. Negative control (NEG), positive control (POS), dexamethasone group (DEX). Lower row: i.n.-sensitised groups. Ovalbumin group (Ova), positive control (Pos), dexamethasone group (Dex).

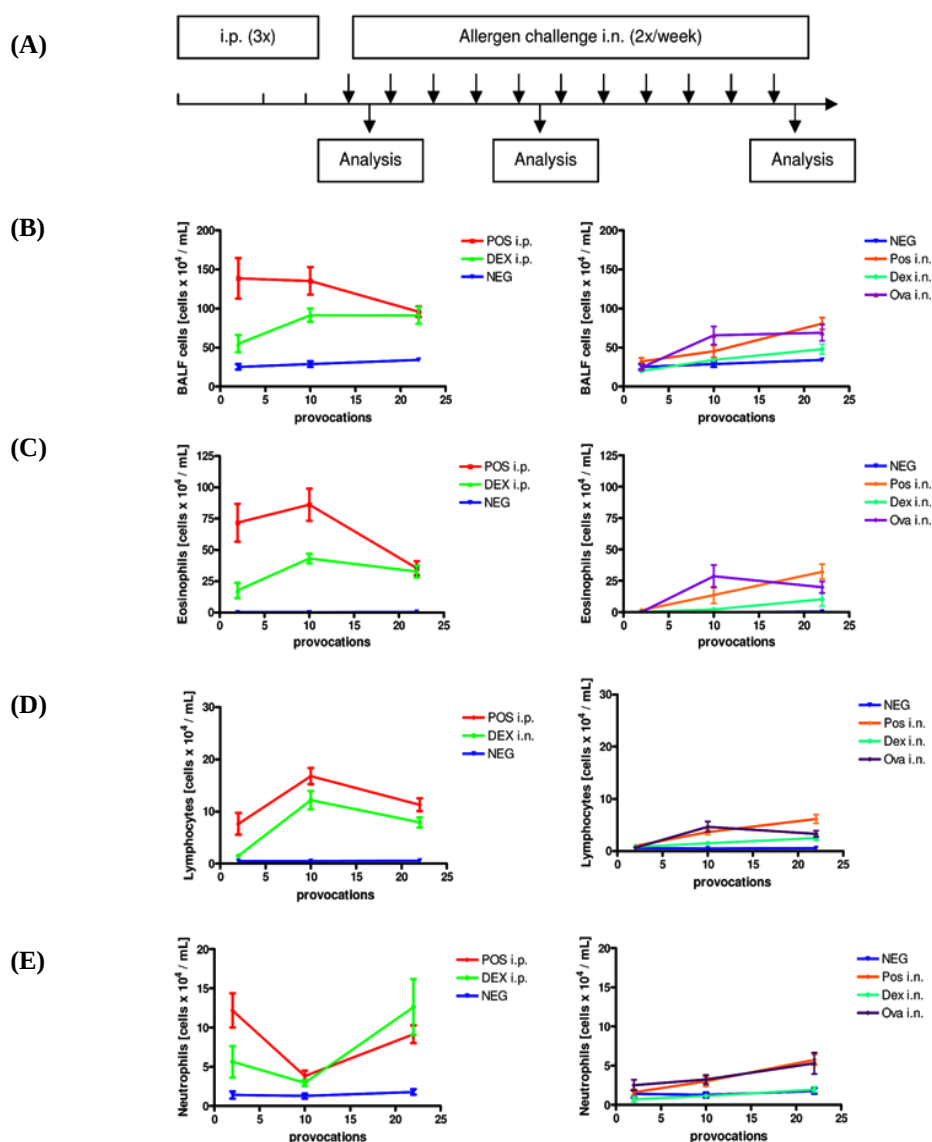


Fig. (2). Protocol of chronic *P. pratense* induced allergic asthma (A). For intraperitoneal sensitisation, BALB/c were injected with r Phl p5/alum on days 1, 14 and 21 or NaCl/alum. Starting at day 28, allergen provocations on two consecutive days/week with extract or ovalbumin were performed in i.p.- and i.n.-sensitized groups. Fifteen and two hours before every provocation dexamethasone was injected i.p., while positive and negative controls received NaCl. Sampling of BALF and tissue was performed after 2, 10 and 22 allergen provocations. **Cellularity of BAL fluid.** Mean and SEM are displayed. Absolute cell counts of BALF (B), eosinophils (C), lymphocytes (D) and neutrophils (E). Left panel: i.p.-sensitized groups. Negative control (NEG), positive control (POS), dexamethasone group DEX. Right panel: i.n.-sensitized groups. Negative control (NEG), positive control (Pos), dexamethasone group (Dex), ovalbumin group (Ova).

asthma. As a biological compound, it is highly variable. For example, the allergenicity is influenced by different natural or environmental factors.

If the plant is stressed, for example by air pollution, allergenicity of the pollen grains raises importantly [67]. Moreover, the allergenicity is further amplified by aggravated occurrence of particles, e.g. diesel exhaust particles, in urban areas [68].

Moreover, due to the natural origin of the pollen grains a contamination of PSG-preparations or lab-made extracts, e.g. with endotoxins, can not be excluded. Endotoxins on the one hand can cause sepsis; on the other hand they are ligands to toll-like receptors and therefore strong immunomodulators [69]. This makes pollen allergen-preparations an allergen source hardly standardisable and therefore probably less recommended in experimental models.

In allergic patients, the allergic reactions are not exclusively mediated by protein allergens: Following the osmotic burst of the pollen grain after contact to the mucosa, not only granules and proteins are ejected, but also lipids. These lipids are originally biological relevant for the directional growth of pollen-tubes [70]; however, pollen grains containing these pollen-associated lipid mediators (PALMs) are *per se* able to activate the mucosal membrane [71]. On DCs, prostaglandine-like compounds called E1 phytoprostanes (PPE1) inhibit the production of IL-12 and thereby promote a Th2 response [67]. Additionally, PALMs are chemo-attractants for eosinophils [72]. The use of standardised pollen extract preparations containing both proteins and lipids, either for induction of allergic inflammation in sensitized mice or especially for respiratory sensitisation, is therefore a method highly reflecting the human disease.

In extract preparations, a variety of major allergens act synergistically. Possibly, at least in some mouse strains, the different major allergens may influence each other in capacity of sensitisation [54].

These observations may let one doubt to which extent adjuvant-free models using extract preparations or natural allergen preparations are really adjuvant-free. Moreover, the standardisation of the allergen preparations requires a careful monitoring, independent from the allergenic organism. Clearly, endogenous adjuvants of the allergen source remain less artificial in the set-up of allergic asthma.

Comparison of Chronic Allergic Airway Inflammation Induced by Timothy-Grass Pollen Allergens: Classical i.p./Alum-Sensitisation vs. Respiratory Sensitisation

In our own group, two different chronic models of allergic airway inflammation are established which use timothy grass allergens. Here, a direct comparison between the outcomes of an i.p.-sensitised rPhl p5/alum model and a model of respiratory sensitisation with intranasal extract challenges in BALB/c mice revealed structural changes in lung tissue as well as changes in cellularity of BALF in both models. In histology, the pattern of remodelling induced by both protocols is comparable in positive controls; however, an expected abrogation of inflammation and remodelling by steroid treatment [73-75] could only be detected in i.n.-sensitised

animals, underlining the dissimilarity of both models (Fig. 1).

One prominent difference was the occurrence of serum IgE: in i.p./alum sensitised animals, huge amounts of serum IgE could be measured, which were not altered by dexamethasone treatment; however, in respiratory sensitised mice sensitisation seems to be independent from IgE (data not shown).

Symptoms of allergic inflammation were seriously more prominent in i.p.-sensitised mice; however, while for example eosinophil counts still showed a clear trend to increase continuously after 11 weeks of extract challenge in respiratory sensitised animals, BALF eosinophilia in i.p.-sensitised animals already decreased (Fig. 2). The corticosteroid dexamethasone inhibited that reduction of eosinophilia, presumably due to tolerance-inhibition [76]. Interestingly, i.n. administered OVA without i.p. sensitisation failed to induce eosinophilia in our hands in contrast to other groups [77].

CONCLUSIONS

Although there are questions remaining about fundamental mechanisms leading to an established chronic asthma, we are nowadays provided with useful models to investigate exactly these questions. Furthermore, we now have the ability to investigate the differences and similarities in acute vs. chronic asthma.

The new improved models of chronic asthma will broaden our knowledge and thereby provide new strategies for preventive or therapeutic interventions.

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ABBREVIATIONS

AHR	=	Airway hyperreactivity
BAL(F)	=	Bronchoalveolar lavage (fluid)
Ig	=	Immunoglobuline
IL	=	Interleukin
OVA	=	Ovalbumin
PALM	=	Pollen-associated lipid mediator
PSG	=	Pollen starch granule
Th T	=	Helper

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