

The relevance of murine animal models to study the development of allergic bronchial asthma

UDO HERZ,¹ USCHI LUMPP,¹ JOAO CASIMIRO DA PALMA,¹ KARLHEINZ ENSSLE,² KIYOSHI TAKATSU,³ NORBERT SCHNOY,⁴ ANGELIKA DASER,¹ ECKART KÖTTGEN,¹ ULRICH WAHN⁵ and HARALD RENZ¹

Departments of ¹Clinical Chemistry and Biochemistry, ⁴Pathology and ⁵Pediatric Pneumology and Immunology, Virchow-Klinikum of the Humboldt-Universität, Berlin, ²Behringwerke AG, Marburg, Germany and ³Department of Immunology, Institute of Medicine Science, University of Tokyo, Japan

Summary Bronchial asthma (BA) develops on the basis of a genetic predisposition and involves a characteristic sequence of changes in immune functions. In the immunopathogenesis, several phases can be distinguished: the initial stage is defined as the development of allergic sensitization. This step is dependent on: (i) T cell activation; (ii) IL-4 production; (iii) IgE synthesis; and (iv) mediator release by effector cells. The second phase of allergic inflammation as a consequence of the T cell dependent sensitization is characterized by IL-5 production and eosinophil activation and recruitment. Airway mucosa remodelling is the consequence of chronic inflammatory processes and represents the final stage of BA. In this article animal models will be discussed with regard to their relevance for these different phases in development of chronic allergic BA.

Key words: airway hyperresponsiveness, allergic inflammation, bronchial asthma, hu-SCID mouse, IgE, murine animal model, SIL-4R.

Introduction

Bronchial asthma (BA) is a chronic obstructive disease of the lower airways and represents a main manifestation of the atopic syndrome together with allergic rhinitis and atopic dermatitis. In asthmatic patients a relationship between genetic predisposition, IgE production, allergen exposure and airway obstruction is well established.^{1,2} Histopathological examination of asthmatic airway and lung tissue reveals the presence of a chronic inflammatory response. Key features include infiltration of mononuclear cells, eosinophils and mast cells.^{3,4} Most of these cells are highly activated. This inflammatory response correlates with the development of increased airway responsiveness (AR). A link between immunologic events, including IgE production, and the state of airway hyper-reactivity is suggested from several epidemiological studies.^{1,2,5,6} These and other results point to a multidirectional dysregulation, where several regulatory systems interact with each other including the immune system, endocrine and neural factors.

Bronchial asthma represents a dynamic disease. The development of the disorder involves a genetic predisposition. Dependent on the allergic phenotype several genetic hot spots were recently identified. For example, allergen-specific IgE production was restricted to certain MHC class II haplotypes and T cell receptor gene

usage.^{7–9} On the other hand, total IgE production was linked to the IL-4 cluster on chromosome 5. More recently, increased AR was linked to a neighbouring part on chromosome 5 and to a region of chromosome 11 (11q13) that includes genes encoding the Fcε-RI.^{10,11} These findings demonstrate that allergic diseases are most likely polygenic diseases and it seems highly unlikely that a single atopic gene responsible for all phenotypes and stages of atopic diseases will be identified.

This complexity can be described in part by the dynamic process of allergic disorders. From an immunological point of view, the asthma cascade is initiated by allergic sensitization (Fig. 1). These include presentation of allergens by APC to T cells, followed by T cell activation and IL-4 production. Further T–B-cell cooperation then controls regulation of IgE production. Interaction of specific IgE antibodies on the surface of effector cells (mast cells, basophils) with allergen triggers the early phase of immediate-type hypersensitivity responses.

These events initiate the development of allergic inflammation. The second phase is characterized by infiltration of eosinophils, release of eosinophil-dependent mediators followed by all signs and symptoms of an inflammatory process. An important linkage between sensitization and inflammation is represented by T cells that secrete mediators not only involved in IgE synthesis but also responsible for eosinophil recruitment, activation and survival.^{12–15}

Like in all other inflammatory disorders tissue destruction occurs and is followed by tissue repair processes. Under optimal conditions complete repair results in restoration of the original tissue function (*restitutio ad inte-*

Correspondence: Harald Renz, Institute of Clinical Chemistry and Biochemistry, Virchow-Klinikum of the Humboldt-Universität, Forschungshaus, Augustenburger Platz 1, 13353 Berlin, Germany.

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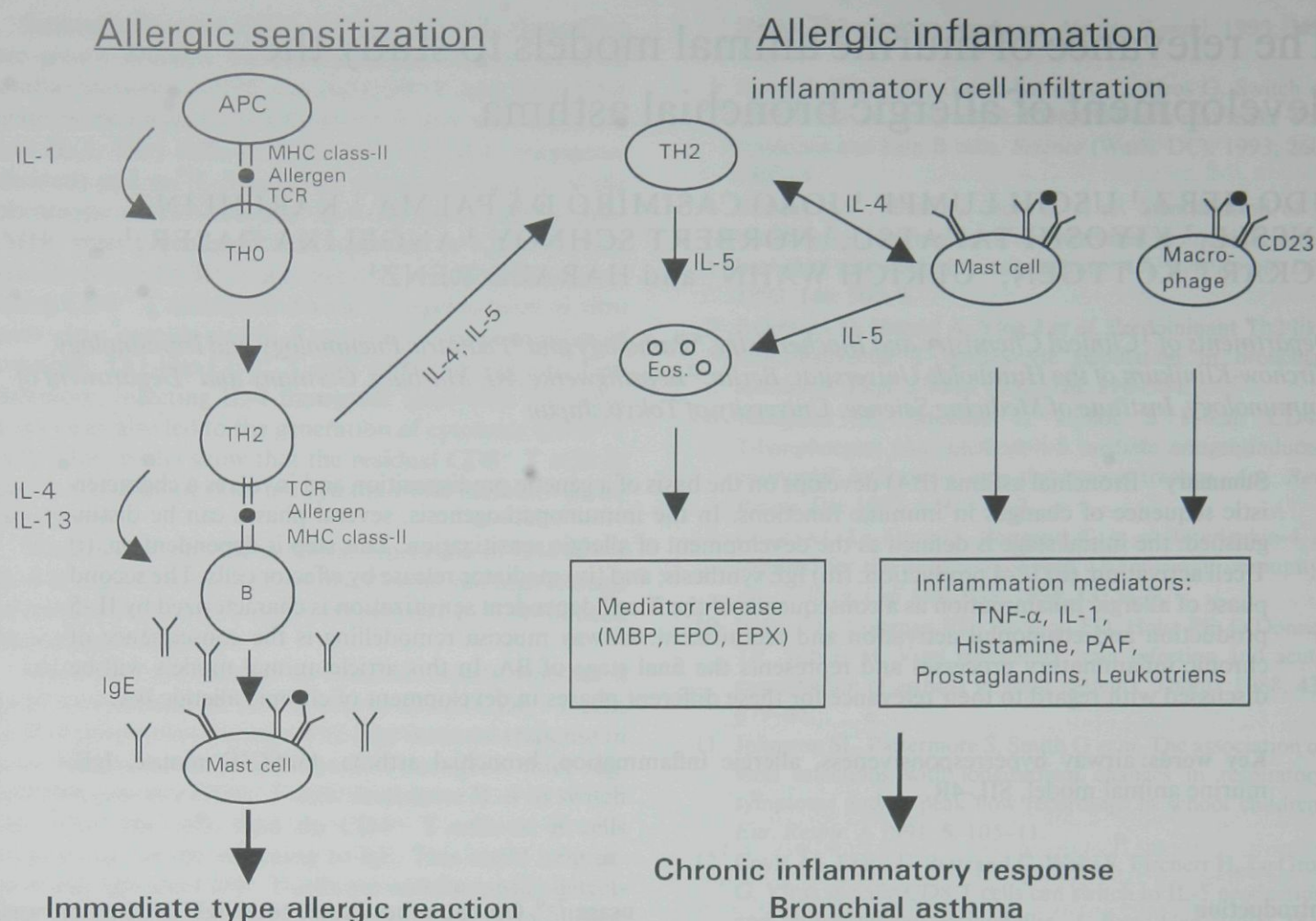


Figure 1 Critical events in development of allergic sensitization and chronic inflammation in human BA.

grum). In particular, this form of tissue repair is achieved in acute and limited inflammatory events. In BA, however, the situation is different. The uncontrolled chronic inflammatory disease eventually results in loss of organ functions due to tissue remodelling. This end stage of the disease is much less understood compared with the events that govern allergic sensitization and inflammation; however, recent results indicate that macrophages, fibroblasts and myofibroblasts play an important part in tissue remodelling.¹⁶⁻¹⁸

This article will discuss, whether the stages in the immunopathogenesis of BA can be mimicked in animal models. Cellular and molecular aspects of the induction and regulation of IgE production, the development of increased AR and the role of allergic inflammation will be described.

Allergic sensitization and IgE production

Mice have been widely used to study the regulation of IgE production under *in vivo* conditions. Many regulatory events in the murine immune system parallel the human immune responses. The availability of inbred mouse strains, transgenic and knock-out mice, and a large variety of mAb add to the attraction of using mouse models to investigate human diseases. We and others have used them to delineate the events that result in local and systemic allergic sensitization. Systemic (i.p.) injection of

allergens such as pollen and animal allergens adsorbed to adjuvant (Al(OH)₃) results in raise of total and allergen-specific antibody titres in high-responder mouse strains.^{19,20}

House dust mite allergens represent important indoor allergens.²¹ Many paediatric and adult patients suffering from allergy show sensitization and clinical symptoms associated with house dust mites. In the natural course of the disease, house dust mite allergens follow food allergens in many patients. To explore the immunological events that control and govern sensitization to house dust mites, mouse models were adapted to this allergen. Since the immunologic response to allergens seems to be genetically predetermined, IgE high- and low-responder mouse strains were analysed for their ability to mount an anti-*Dermatophagoides pteronyssinus* (Der p) IgE/IgG1 antibody response. The results revealed that C57BL/6 and CBA mice are high-responder strains, whereas BALB/c mice are characterized as low-responder animals (Table 1), which confirms results obtained by other groups.²²⁻²⁴ It becomes apparent that, following immunization to allergens, each individual mouse strain has a unique response pattern. For example, BALB/c mice are known as IgE-high responders to ovalbumin (OVA), whereas the same mice are poor responders to Der p.²² These mouse models represent excellent model systems to delineate the genetic and immunological basis for such differences.

Table 1 Allergic sensitization to house dust mite allergen (Der p) in different mouse strains

Mouse strain	MHC haplotypes	Sensitization to	Baseline AR (ES ₅₀) ¹	AR after sensitization (ES ₅₀)	Immediate-type allergic reaction ²
C57BL/G	H-2 ^b	Der p	3.1 ± 0.8	1.9 ± 0.7 <i>P</i> < 0.001	High
CBA	H-2 ^k	Der p	1.8 ± 0.7	1.2 ± 0.4 NS	Intermediate
BALB/c	H-2 ^d	Der p	1.8 ± 1.4	2.5 ± 0.7 NS	Low

Mice were sensitized by i.p. injection of Der p (containing 5 µg Der p1; ALK-Scherax GmbH, Hamburg, FRG) on days 1 and 14. On days 22 and 23 mice received two consecutive local allergen challenges via the airway by aerosolization of Der p extract (equivalent to 7 µg Der p1) diluted in 7 mL PBS for 20 min as previously described.²⁰

Airway smooth muscle responsiveness was assessed by electrical field stimulation as previously described.⁴¹

Values are mean ± s.d. of electrical frequencies that resulted in 50% of maximal smooth muscle contractility (ES₅₀).

Immediate-type allergic reaction were classified on the basis of elevated IgE/IgG1 antibody titres and development of immediate-type cutaneous hypersensitivity reactions.

The T cell-mediated process of allergic sensitization comprises several cognate and non-cognate interactions with the immune response. The first step is the uptake and internalization of environmental allergens by APC (Fig. 1). APC phagocytose and process allergens into small peptide fragments. They present these allergenic peptides as T cell epitopes on MHC class II molecules to CD4⁺ T cells which recognize them using their T cell receptor (TCR). Each T cell expresses one unique TCR phenotype on the cell surface. The individual composition of different TCR elements defines the antigen-allergen specificity of a T cell. For example, in BALB/c mice, OVA sensitization was associated with a preferential expansion of Vβ8.1/8.2 T cells in peribronchial-draining lymph nodes.²⁵ Transfer of Vβ 8.1/8.2 T cells from sensitized mice stimulated an anti-OVA IgE response in naive syngeneic recipients.²⁶ In contrast transfer of CD8⁺ cells from OVA-sensitized mice and rats failed to initiate or induce IgE production,^{27–29} or even inhibit immediate-type hypersensitivity reactions.^{24,30} This negative function of CD8⁺ T cells was attributed to increased IFN-γ production by these cells. In addition another T cell population, γ/δ was also found to inhibit these responses.³⁷ These results confirm that allergic sensitization and IgE synthesis are T cell dependent events.

The MHC class II molecule, allergenic peptide and the TCR assemble in a trimolecular complex and initiate T cell activation. In addition to this cognate interaction between APC and T cells, other non-cognate stimuli are required to fully activate naive T cells, for example IL-1 released from activated APC or the interaction between B7 and CD28 on T cells.³¹ This process results in full activation of allergen-specific T cells and promotes differentiation into IL-4/IL-5-producing type 2 T cells. IL-4 induces ε-germline transcription in B cells but is not sufficient to stimulate IgE protein synthesis. Further contact-mediated, co-stimulatory interactions are necessary to complete IgE isotype switching, such as an interaction between CD40 ligand (which is transiently expressed on activated T cells) and CD40 (constitutively expressed on B cells).³²

Murine studies have indicated that IL-4 plays a key role in IgE production *in vivo*, because parasite-infected mice treated with neutralizing anti-IL-4 antibodies failed to produce IgE.³³ Furthermore, in IL-4 knock-out mice,

serum IgE was not detectable, and nematode infection could not restore IgE antibody responses in such mice.³⁴ On the other hand, the serum IgG1 levels in IL-4 knock-out mice were reduced, but only to one-third or one-half of the control level, suggesting that in addition to IL-4 other cytokines are involved in the isotype switch to IgG1. One of the candidates is IL-6, since a selective increase in serum IgG1 levels was observed in IL-6 transgenic mice.³⁵ In humans there is also a second cytokine, IL-13, known to promote IgE production. In contrast, the biological activities of IL-13 in mice seem to be more restricted, because it does not act on murine B cells.³⁶

These findings lead to an important question regarding the regulation of immediate hypersensitivity responses. Is the presence of a T cell dependent allergen-specific IgE response sufficient to elicit these responses? To analyse the relevance of IgE production triggering immediate-type allergic responses two different functional read-out systems will be used. The first is the elicitation of positive immediate-type skin test responses, and the second the assessment of airway smooth muscle reactivity.

Development of immediate cutaneous hypersensitivity in Der p-sensitized mice

As previously reported,³⁸ OVA-sensitized BALB/c mice responded to i.d. injected allergen solution with development of wheal formation. The kinetics of these responses followed the time course of human skin prick test reactions and correlated with the presence of allergen-specific IgE/IgG1 antibodies. Histological examination of skin reactions revealed that these responses were associated with marked mast cell degranulation. In mice, the development of immediate-type cutaneous hypersensitivity (ICHS) reactions may not only be triggered by crosslinking of IgE antibodies bound to Fcε-R1 on the surface of murine mast cells. In addition, an alternative mechanism involves binding of IgE (and IgG1) antibodies to Fcγ-RII and Fcγ-RIII on mouse mast cells.³⁹ These results provide evidence for a functional role of allergen specific IgE/IgG1 antibodies to elicit ICHS reactions that closely mirror the human type I allergic-response.

Based on these results we analysed whether Der p-sensitized C57BL/6 mice would also develop

immediate-type skin reactions to i.d.-injected Der p solution. Animals were sensitized to house dust mite extract (Der p) by i.p. injection on days 1 and 14. The allergen was adsorbed to $\text{Al}(\text{OH})_3$ and calibrated based on the concentration of Der p 1 ($5 \mu\text{g}$ Der p 1/injection). On days 22 and 23 animals received two consecutive allergen challenges of aerosolized Der p extract (containing $7 \mu\text{g}$ Der p 1/challenge) delivered via the airways. To visualize increased vascular permeability and leakage upon mast cell degranulation and mediator release, 0.5% Evans blue solution was i.v. injected before skin testing followed by i.d. injection of test solutions. The response to Der p (500 – 5000 standard quality (SQ) units/injection equivalent to 35 – 350 ng Der p 1/injection) was compared with a positive (compound 48/80) and negative (PBS) control solution. An example of a positive reaction is presented in Fig. 2. Only Der p-sensitized mice developed positive ICHS reactions to Der p that correlated with an allergen-specific lymphocyte response and an increase in serum IgE/IgG1 antibody titres (Table 2).

Development of increased airway responsiveness in allergen-sensitized mice

In recent years several techniques have been developed that allow measurement and analysis of AR in mice under *in vivo* and *in vitro* conditions.^{40,41} These read-out systems were used to study the development of increased AR in allergen-sensitized mice. It is well established that the immediate effects of airway obstruction are related to degranulation of effector cells including mast cells and eosinophils. According to this concept, allergen exposure leads to crosslinking of cell-surface immunoglobulins which triggers the release of preformed and rapidly

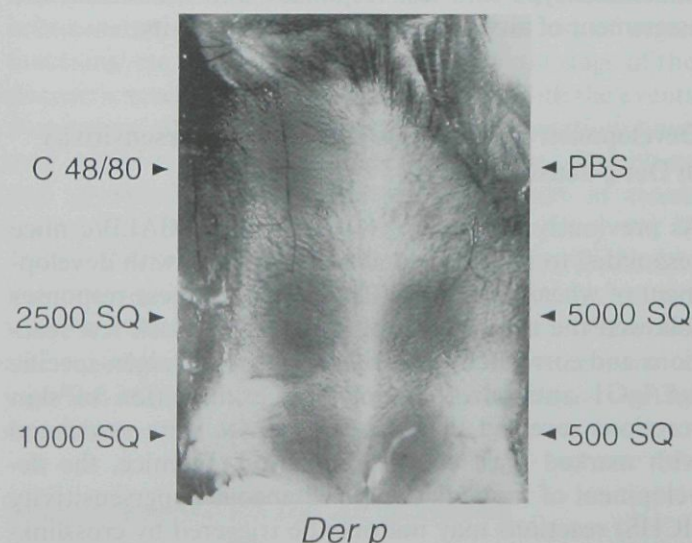


Figure 2 C57BL/6 mice were sensitized to Der p (containing $5 \mu\text{g}$ Der p 1/injection) (ALK, Ry, Denmark) adsorbed to $\text{Al}(\text{OH})_3$ by i.p. injection on days 1, 14 and 21. Skin tests were performed on day 26. Before skin testing, $150 \mu\text{L}$ of a 0.5% Evans blue (v/v) solution was injected intravenously. Intracutaneous skin tests were performed with PBS, compound 48/80 ($5 \mu\text{g}/\text{mL}$) and Der p in concentrations as indicated. All solutions were injected i.d. in a volume of $50 \mu\text{L}$. After 20 min vascular leakage was monitored on the reversed skin.

Table 2 Systemic sensitization results in IgE production, allergen-specific lymphocyte proliferation and immediate-type cutaneous hypersensitivity (ICHS)

	Systemic sensitization of C57BL/6 mice	
	$\text{Al}(\text{OH})_3$	Der p + $\text{Al}(\text{OH})_3$
Total IgE (ng/mL)	108 ± 90	327 ± 110
Lymphocyte proliferation to Der p SI	0.7	5.0
Positive ICHS reactions to Der p	0/8	18/22

Total IgE was measured by ELISA; indicated are changes in total IgE (mean $\pm$ s.d.) measured before sensitization and on day 24; baseline IgE was $160 \pm 30 \text{ ng}/\text{mL}$.

Splenic lymphocytes were prepared from sensitized ($n = 4$) and non-sensitized animals ($n = 4$); allergen specific lymphocyte stimulation was assessed in a proliferation assay of mononuclear cells stimulated with and without Der p ($10 \mu\text{g}/\text{mL}$; 4 days); cells were pulsed with $1 \mu\text{Ci}$ $^3\text{H}[\text{TdR}]$ 24 h prior to cell harvesting; mitogen stimulation was assayed in parallel. SI = cpm sample/cpm medium contrast.

Immediate cutaneous hypersensitivity responses were analysed on day 27 as described previously.³⁸ Values are the numbers of animals with the positive wheal formation after i.d. application of Der p; there were no positive reactions to PBS and all animals developed positive wheal formations to compound 48/80.

synthesized mediators by mast cells including histamines, prostaglandins, leukotrienes, and platelet activating factor. Increased concentrations of these and other mediators have been detected in bronchoalveolar lavage (BAL) fluids of asthmatic patients.^{42,43}

We used the Der p sensitization model to delineate minimal requirements for induction of a state of increased AR in mice. We hypothesized that stimulation of a (systemic) allergen-specific T cell response that leads to increased production of allergen-specific IgE/IgG1 antibodies would be sufficient to mount such responses. These antibodies would then bind to available airway mucosal mast cells. Local allergen challenge would then provide the natural ligand to trigger the release of mediators responsible for constriction of airway smooth muscle.

For these studies we used the technique of electrical field stimulation of tracheal smooth muscle. It was previously shown that this method triggers smooth muscle constriction via neural and cholinergic mediators, since the response was abolished by tetrodotoxin and atropine. Furthermore, it was demonstrated that this type of response correlated with increased *in vitro* release of acetylcholine by tracheal segments.⁴⁴ Thus, the response to electrical field stimulation mimics several important events observed in airway smooth muscle constriction.

We first determined the baseline contractility of tracheal smooth muscle segments (TSM) prepared from non-sensitized C57BL/6 mice. For each animal a dose-response curve was established from which ES50 values were calculated. The ES50 is defined as the electrical frequency resulting in 50% of maximal constriction of TSM. The slope of these dose-response curves correlated well with the dose-response curves observed in another mouse model.⁴¹ Non-sensitized C57BL/6 mice did re-

spond to electrical stimulation with an ES₅₀ of 3.1 ± 0.8 Hz (Fig. 3). Systemically-sensitized C57BL/6 mice responded with the same degree of AR as indicated by an ES₅₀ of 2.8 ± 0.7 Hz. These results demonstrate that systemic sensitization and IgE and IgG1 production alone were not sufficient to alter the degree of AR. An explanation for this finding might be that, although IgE and IgG1 were present on the surface of effector cells, in the absence of crosslinking allergen mediator release via signalling through IgE and IgG1 receptors does not occur. To prove the necessity of a local allergen challenge, AR was analysed in mice with and without a local allergen challenge prior to analysis. For this purpose mice were exposed to an aerosol of Der p (containing 7 µg of Der p 1 diluted in 7 mL PBS and aerolized over 20 min). In non-sensitized mice two consecutive allergen challenges did not alter the AR pattern (2.8 ± 0.7). Only when sensitized C57BL/6 mice were locally challenged development of increased AR was observed as indicated by a decrease in ES₅₀ values to 1.9 ± 0.7 Hz (Fig. 3). These results illustrate that increased AR was dependent on systemic sensitization and delivery of local allergen challenges via the airways.

To examine whether such phenotype of increased AR is associated with an inflammatory cell infiltrate, histopathologic analysis was performed. A small infiltrate of lymphocytes was observed around small blood vessels (data not shown). There was no significant increase of eosinophils. These data indicate that systemic sensitization stimulates an allergen-specific IgE/IgG1 response in susceptible mice. These IgE and IgG1 antibodies are of functional activity as revealed by development of immediate-type skin test responses and increased AR following local allergen challenge. In virus-free animals that were housed under pathogen-free conditions, systemic sensitization was not associated with an inflammatory response in the lung. Even after two consecutive allergen challenges delivered through the airways, only minimal cell influx was observed after 24 h. Such changes were mainly restricted to an influx of mononuclear cells into the airway mucosa. A marked infiltration of eosinophils was not detected in this Der p animal system. Therefore,

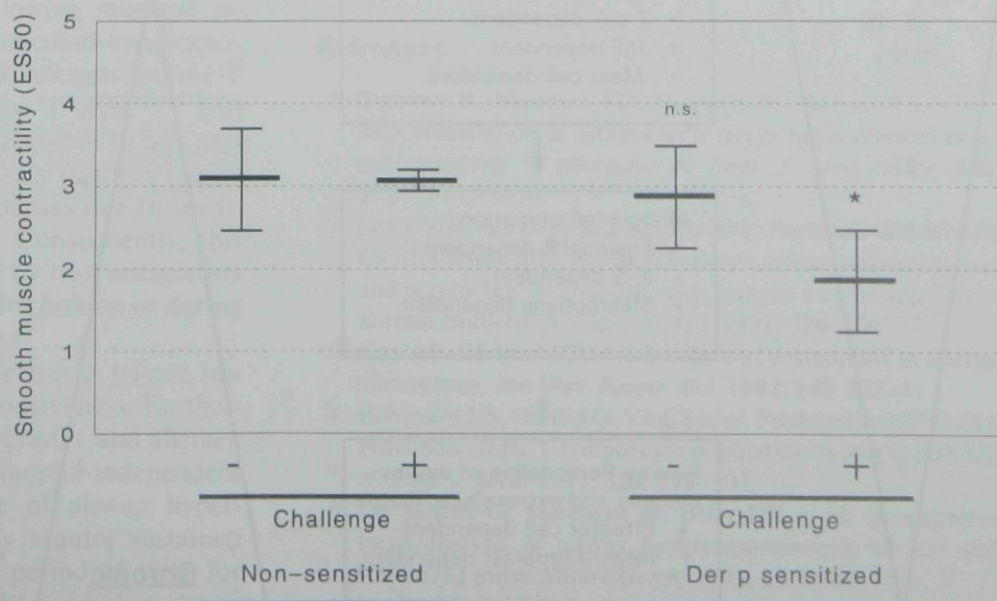
this mouse model reflects a state of allergic sensitization and illustrates that sensitization together with local allergen provocation is sufficient to trigger immediate-type hypersensitivity responses at the effector organ but not to reach an immunological process like ongoing inflammation.

The humanized SCID mouse (hu-PBMC) as a model for *in vivo* studies of human (hu)-IgE regulation

As IL-4 is the key cytokine in the allergic sensitization cascade, therapeutic strategies have targeted this cytokine in the human and the mouse system. In addition to anti-IL-4, sIL-4R modulates many IL-4-mediated T and B cell functions.^{45–47} Recently we showed that administration of murine recombinant sIL-4R inhibited the production of total and allergen-specific IgE production in OVA-sensitized BALB/c mice, and this correlated with normalization of airway hyper-reactivity.⁴⁸ To test whether human sIL-4R could inhibit human IgE production under *in vivo* conditions we developed a hu-SCID mouse model with peripheral blood mononuclear cells from atopic patients (hu-atopic PBMC). Human PBMC purified from Der p-sensitized donors (anti-Der p IgE 1.9–860 ng/mL), were transferred in the presence of Der p allergen into the peritoneal cavity of SCID mice ($3.6\text{--}7.5 \times 10^7$ PMBC/mouse). Eight days later, SCID mice received an additional boost with Der p allergen by i.p. injection. Total and allergen-specific hu-IgE antibody titres were analysed by fluorescence-enzyme-immunoassay (FEIA) on day 22.

Two strategies for sIL-4R treatment were compared. A prophylactic approach in which hu-PBMC SCID mice were treated with human sIL-4R (150 µg/mouse/application) in parallel with cell transfer and allergen boost on days 1–3 and again on days 7–9. A therapeutic approach in which hu-PBMC SCID mice were treated daily with human sIL-4R on days 9–13 only. As a control, hu-PBMC SCID mice were treated in parallel with murine sIL-4R (150 µg/mouse/application) because this protein does not interact with human IL-4.

Figure 3 C57/BL6 mice were sensitized to Der p as described in Figure 2. Airway responsiveness was measured by *in vitro* stimulation of TSM segments. Indicated are mean $\pm$ s.d. of electrical frequencies that resulted in 50% of maximal contractility (ES₅₀). Certain animals received two consecutive local allergen challenges via the airways delivered by aerosolization of Der p extract (containing 7 µg Der p 1/aerosolization). *Indicates significant difference ($P < 0.05$) between non-sensitized mice and Der p-sensitized animals, all of them receiving two consecutive local allergen challenges.



Prophylactic treatment of hu-PBMC SCID mice with human sIL-4R resulted in reduced levels of hu-IgE antibody titres (4 and 66%) in comparison with hu-PBMC SCID mice that received murine sIL-4R (Table 3). In contrast, therapeutic treatment with human sIL-4R did not influence human IgE production in this animal model system. We conclude that an established IgE immune response is not dependent on IL-4 production and, therefore, therapeutic treatment with sIL-4R cannot decrease hu-IgE antibody titres. In contrast, if sIL-4R is administered at an early stage of IgE induction the drug suppressed the production of allergen-specific IgE to a large extent (Fig. 4).

The role of IL-5 in a model of allergic sensitization

Eosinophils represent a hallmark in allergic airway inflammation. IL-5 is a cytokine regulating many functions of eosinophils.⁴⁹ Several studies showed that sensitization of mice can result in the recruitment of T cells into the lung.^{20,50} A possible mechanism to initiate allergic inflammation processes in the lung could be the release of IL-5 by allergen-specific, activated T cells. This may act in a synergistic fashion with the release of other pro-inflammatory cytokines such as TNF- α and IL-1. In the presence of these and other cytokines, epithelium cells then up-regulate adhesion molecules (VCAM-1, ICAM1) and further support the recruitment of eosinophils. Recently, it was suggested that eosinophil recruitment alone could contribute to increased airway reactivity because intra-nasal administration of IL-5 to non-sensitized BALB/c mice resulted in an increase of eosinophils in BAL that correlated with increased airway hyper-reactivity and mucosal exudation. In this model *in vitro* AR was measured following methacholine challenges in an allergen-independent fashion.⁵¹ In addition, several

Table 3 Prophylactic but not therapeutic treatment with hu-sIL-4R inhibits hu-IgE production in hu-PBMC SCID mice

Treatment	Atopic donor	Treatment of hu-PBMC SCID mice with	Total hu-IgE (ng/mL)
Prophylactic	01	mu-sIL-4R	147.6
		hu-sIL-4R	6.4 (4%)
	02	mu-sIL-4R	76.3
		hu-sIL-4R	50.4 (66%)
Therapeutical	10	mu-sIL-4R	56.9
		hu-sIL-4R	52.9 (93%)
	11	mu-sIL-4R	49.6
		hu-sIL-4R	45.5 (92%)
	12	mu-sIL-4R	72.7
		hu-sIL-4R	80.4 (111%)

Human PBMC derived from atopic donors (total IgE $\geq$ 5000 ng/mL) were injected in combination with Der p into the peritoneal cavity of SCID mice. Eight days later hu-PBMC SCID mice received an additional boost with Der p.

studies reported the effectiveness of anti-IL-5 or anti-VCAM I antibody treatment to inhibit eosinophil recruitment and allergen-induced airway hyper-responsiveness.⁵²⁻⁵⁴ Based on these results we examined whether anti-IL-5 administration to Der p-sensitized C57BL/6 mice would prevent development of airway hyper-responsiveness in our model system.

C57BL/6 mice were systemically (i.p.) sensitized to Der p adsorbed to Al(OH)₃ on days 1 and 14. In parallel, mice were treated with an anti-IL-5 mAb at a dose of 500 μ g/injection administered on days 1 and 13. Mice were analysed on day 22. Sensitized mice treated with and without anti-IL-5 showed elevated serum IgE antibody titres that were paralled by *in vitro* increased IL-4 production from mitogen-stimulated splenic lymphocytes (273 $\pm$ 54 pg/mL in sensitized C57BL/6 and 472 $\pm$ 83 pg/mL in anti-IL-5-

The cascade of bronchial asthma

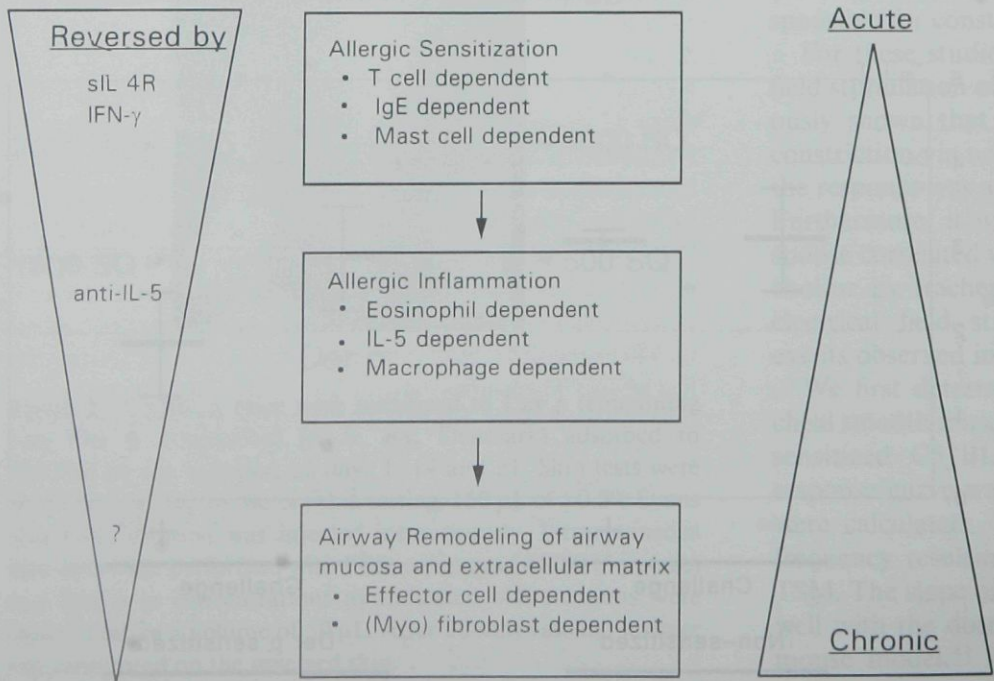


Figure 4 Hypothetical concept of human BA.

treated mice). In addition, both groups of mice developed positive skin test reactions after i.d. injection of allergen solution (67% of sensitized untreated mice and 75% of sensitized mice treated with anti-IL-5). Anti-IL-5-treated mice showed a tendency to normalization of increased AR in comparison with sensitized untreated mice. However, these change did not reach statistical significance (1.9 ± 0.8 Hz in sensitized mice compared with 2.7 ± 1.2 Hz in sensitized mice treated with anti-IL-5).

These results indicate that the development of an allergic immune response was not influenced by treatment with anti-IL-5 antibody, suggesting that, in contrast with IL-4, IL-5 is not involved in developing type 2 T cell responses and does not play a critical role in development of IgE-dependent allergic responses. IL-4 plays a central role in development of immediate-type hypersensitivity responses because treatment with recombinant sIL-4R prevented allergic sensitization (Fig. 4). In contrast, when the activity of IL-5 was neutralized in models of allergic inflammation, the induction of such inflammatory responses as well as the development of IgE-independent, but eosinophil-dependent, airway hyper-reactivity was prevented.^{55,56}

Conclusions

The murine models of Der p sensitization illustrate that the development of an immediate-type hypersensitivity response requires susceptible mouse strains. The genetic components that determine 'susceptibility' have not as yet been delineated. A number of different genetic determinants may be involved, including the MHC class II haplotype, that may be able to present Der p-derived peptides in a fashion resulting in appropriate T cell activation via interaction with TCR components and other co-stimulatory molecules. The identification of IgE high- and low-responder strains opens the opportunity for further studies aimed at identifying and characterizing genes that play a critical role in development of the allergic immune response and phenotype.

The development of allergic sensitization is T cell dependent.⁵⁷ We identified the following minimal requirements for induction of *in vivo* immediate-type hypersensitivity responses: (i) activation of allergen-specific T cells; (ii) production of IL-4 by these T cells;^{58,59} (iii) IL-4-dependent immunoglobulin class switch to IgE; and (iv) local allergen exposure as a necessary factor to trigger immediate-type hypersensitivity responses due to mediator release by effector cell subsets. Consequently, this phase of the disease can be inhibited by IL-4 antagonists or IFN- γ if administered in a preventive fashion or during early stages of the sensitization process.

These requirements are also sufficient to trigger immediate events of airway hyper-responsiveness. Furthermore, this type of increased AR is IgE/IgG1 and allergen dependent but develops in an eosinophil-independent fashion. It is likely that this type of airway hyper-responsiveness is transient and may require sustained local allergen exposure over a long period of time for chronic airway constriction. It is well known that a state

of increased AR precedes the development of clinically relevant asthmatic symptoms in many patients. This state of airway hyper-reactivity may be related to the observation of increased AR in this model of allergen-sensitized mice. Under the experimental conditions explored so far, the second stage of the disease, development of allergic inflammation, has not been reached. These observations would also support the concept that BA represents a multi-stage disease that is initiated by immunological events of allergic sensitization. Infiltration of (activated) eosinophils in addition to the presence of mononuclear cells represent the hallmark of allergic inflammation.^{51,60,61}

Furthermore, this concept would suggest in an indirect fashion that additional trigger factors are required to stimulate the inflammatory response. Whether prolonged (local) allergen exposure is sufficient to mount such a response needs to be determined. On the other hand, it is also possible that factors or agents other than allergens are also able to push the immune system from sensitizing events towards the inflammatory stage. Candidates include certain viral antigens, environmental factors, toxic agents and others.

Presently, most mouse models, including the Der p model presented here, cover the stage of allergic sensitization. Identification of additional trigger factors will result in the development of animal model systems that mirror the immunologic situation in human BA more closely including the stage of chronic inflammation followed by airway remodelling. Such animal model systems could not only be used to dissect the (immuno)pathogenesis of asthma but would also offer the opportunity to study the effects of immunomodulatory agents to alter the course of the disease.

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