

The Impact of the Route and Frequency of Antigen Exposure on the IgE Response in Allergy

A. Nelde^a M. Teufel^b C. Hahn^b A. Duschl^a W. Sebald^a E.B. Bröcker^b
S.M. Grunewald^b

^aBiozentrum, Physiologische Chemie II, ^bKlinik und Poliklinik für Haut- und Geschlechtskrankheiten, Universität Würzburg, Würzburg, Deutschland

Key Words

Allergy · Animal model · Antibodies · IgE · Allergic sensitisation · Airway

Abstract

Background: Knowledge of the factors which control IgE production is essential in order to understand the pathogenesis of immediate hypersensitivity reactions. We have studied the extent to which the route and frequency of antigen application as well as different antigen amounts may influence IgE synthesis. **Methods:** We established sensitisation protocols in BALB/c mice, in which various doses of ovalbumin (Ova) were applied via intranasal, epicutaneous or intraperitoneal routes. Ova-specific antibodies were measured by ELISA. After 6 weeks of sensitisation, anaphylactic shock was measured following intravenous challenge with Ova. In addition, bronchoalveolar lavages were performed in intranasally sensitised mice. **Results:** We were able to show that the most efficient IgE production was achieved by long-term antigen application via the airways, leading to local allergic airway pathology. The epicutaneous route of antigen application also induced very high IgE titres, while intraperitoneal sensitisation led to significantly

lower IgE levels. After intraperitoneal sensitisation, IgE synthesis was best induced by increasing the frequency of antigen application, but not by increasing the amount of antigen. In all groups of mice, Ova-specific IgE antibodies were high enough to induce systemic allergic symptoms leading to anaphylactic shock. The severity of shock correlated with the amount of specific IgE. **Conclusions:** Taken together, our results demonstrate that antigen application via the airways or skin induces IgE synthesis more efficiently than via the intraperitoneal route. Few exposures with high-dose antigen are less efficient than multiple exposures with low doses. Our finding that both the route and the frequency of antigen application strongly influence IgE synthesis may help to understand how environmental antigens lead to allergic sensitisation.

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Introduction

The induction of an antigen-specific IgE response is a prerequisite for the development of immediate hypersensitivity reactions. The allergic response is driven by CD4⁺ T helper (Th) 2 cells, whose activation and differentiation

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Correspondence to: Dr. Susanne Grunewald
Department of Dermatology, University of Würzburg, Josef-Schneider-Strasse 2
D-97080 Würzburg (Germany)
Tel. +49 931 201 2710, Fax +49 931 201 2700
E-Mail grunewald-s.derma@mail.uni-wuerzburg.de

arise out of the interaction of many cellular and molecular factors. The activation and expansion are accompanied by differentiation from the pluripotent Th0 state towards the Th1 or Th2 phenotype, which are distinguished by their pattern of cytokine production. Th1 cells produce IL-2, IFN- γ and TNF- α , and elicit IgG2a antibody production in mice as well as other cellular responses. Together, they promote defence against organisms entering the intracellular compartment [1, 2]. In contrast, Th2-derived IL-4 and IL-13 induce germline transcription in mice at the C ϵ and C γ 1 exons, which promotes isotype switching to IgE and IgG1 [1]. In addition to IL-4 and IL-13 production, CD4⁺ Th2 cells express CD40 ligand after antigen encounter and T cell receptor (TCR) activation. Th2 cells can therefore provide both of the signals necessary for IgE production to B cells [3].

Genetic factors predispose some individuals to mount Th2 responses after antigen stimulation, which subsequently induces IgE synthesis. Genome-wide screens and linkage analysis have identified polymorphisms in candidate genes which are likely to contribute to allergic pathogenesis. Non-specific tendencies to produce high IgE levels are linked to 5q31–33, a locus that encodes multiple Th2 cytokines [4]. Clinical atopy and the production of allergen-specific IgE have also been linked to 11q12–13 and may be related to polymorphisms of the high-affinity IgE receptor β chain [5, 6]. As with other antigens, responses to allergens are modulated by the inheritance of particular major histocompatibility class (MHC) II alleles or TCR variable region repertoires.

Environmental factors also play an important role in IgE production, since monozygotic twins have asthma concordance rates of less than 20% [7]. This is probably due to differences in antigen exposure, since the cumulative inhaled dose of sensitisers such as dust mite allergen over many years is directly related to the likelihood of developing allergen-specific IgE and asthma [8]. Environmental factors are determined not only by the cumulative dose and the duration of antigen exposure but also by the nature and structure of the antigens. The major allergens have structural and functional properties that promote Th2 responses, explaining why not all inhaled substances elicit an allergic response. The mechanism for this phenomenon was provided by a study showing that changes in ligand structure and density can bias T cell responses [9]. The intensity of the interaction between the antigenic peptide-MHC class II complex and the TCR has been shown to influence the differentiation into type 1 versus type 2 T cells by delivering different signals via the TCR. Complexes interacting strongly with the TCR favour

type 1 responses, whereas weak interactions prime type 2 responses [10]. In addition, some allergic proteins are toxic to the respiratory epithelium and can actually disrupt tight junctions [7]. Others have been shown to directly modulate host immune functions by specific modification of immunocyte receptors, including proteolysis of CD23 and CD25 [7].

Microenvironmental factors in the tissue where the immune response takes place may also control IgE synthesis. After antigen exposure, the induction of an immune response requires uptake and processing by antigen-presenting cells (APCs), which then present peptide fragments to specific T cells. The type of APC, expression of costimulatory molecules and secretion of cytokines can all influence the phenotype of the resultant T cell response. APCs vary in their ability to induce Th1 or Th2 responses. Macrophages tend to favour the induction of Th1 cells, whereas dendritic cells and B cells may induce Th2 responses [11–13]. This results in various immune responses, due to the presence of different local APC populations.

Since antigen-dependent factors as well as the cellular microenvironment of the individual tissues of first antigen exposure may impact on allergic sensitisation, we studied in an animal model the extent to which the route, dose and frequency of antigen application influence IgE synthesis.

Materials and Methods

Mice

Female BALB/c mice between 8 and 12 weeks of age were purchased from Charles River (Sulzfeld, Germany). The animals were kept in a conventional animal facility and maintained on ovalbumin (Ova)-free diets.

Allergic Sensitisation Procedures

Intraperitoneal Sensitisation. Groups of mice were immunised by single or multiple intraperitoneal injections with 200 μ l of solution containing various amounts of Ova in phosphate-buffered saline (PBS) (grade V; Sigma, Deisenhofen, Germany) or PBS alone. The individual sensitisation course is indicated in table 1.

Intranasal Sensitisation. Mice were anaesthetised by an intraperitoneal injection of a mixture of ketamine and xylazine, then 50 μ g of Ova in a 50 μ l volume of PBS or PBS alone were administered by intranasal inoculation (table 1).

Epicutaneous Sensitisation. Mice were anaesthetised as described above and the back was shaved with an electric clipper. Then, 100 μ l of a PBS solution containing 50 μ g of Ova or PBS alone was applied to a 1 cm² sterile patch. Subsequently, the patch was applied to the shaved skin, fixed with a bio-occlusive dressing and secured with an elastic bandage. Patches were left on for 48 h and then removed. Each sensitisation course was repeated at the same skin site every week for a total of 6 weeks (table 1).

Table 1. Allergic sensitisation procedures

Route of sensitisation	Immunisations	Day	Antigen doses μg	Total amount of antigen, μg
Intraperitoneal	1	0	10	10
	1	0	100	100
	2	0, 21	100, 500	600
	6	0–42	50 every week	300
	20	0–42	15 every 2nd day	300
Intranasal	6	0–42	50 every week	300
Epicutaneous	6	0–42	50 every week	300

Table 2. Intranasal, intraperitoneal and epicutaneous sensitisation led to systemic anaphylaxis

Shock criteria	No Ova	Ova intraperitoneal				Ova epicutaneous	Ova intranasal
		1 $\times$	2 $\times$	6 $\times$	20 $\times$		
Intensity of blueing ¹	0 ²	1	1	1	1	1	1
Spontaneous activity ³	0	1	1.5	1.5	2	1.5	2
Responsiveness to external stimuli ⁴	0	1	1.5	2	2	1	2

¹ Scoring scale: no = 0; blue = 1.² Mean score rate in one group (n = 5).³ Scoring scale: running around = 0; sitting passively = 1; lying = 2.⁴ Scoring scale: running away upon touching = 0; slight reaction = 1; no reaction = 2.

Serum Antibody Titres

Anti-Ova IgE and IgG subclasses were measured by ELISA. Plates were coated for 6 h at 37°C with 100 μl of 0.1 M NaHCO₃ containing 100 μg of Ova/ml. Then the plates were blocked for 2 h at 37°C with 200 μl of 3% bovine serum albumin (BSA) in PBS. The plates were washed and 100 μl of twofold serum dilutions with PBS containing 1% BSA were applied overnight at 4°C. The amount of bound antibody was analysed using horseradish peroxidase-conjugated antibodies against mouse heavy chain classes. Rat monoclonal antibodies against mouse IgE and IgG1 were from Pharmingen (Hamburg, Germany) and goat antisera against mouse IgG2a and IgG2b were from SBA (Biozol, Eching, Germany). The plates were read in a microplate autoreader (Dynatech MR5000, Denkendorf, Germany) at 405 nm. Serum titres for all antibodies are expressed as the reciprocal value of the serum dilutions, which were 1.5 times greater than the background optical density (OD₄₀₅) of non-immunised mice. The quality of ELISAs and detection antibodies was controlled using a laboratory standard serum pool, which was applied to each ELISA plate and collected 20 days after sensitisation with Ova.

Systemic Anaphylaxis

Anaphylactic shock experiments were performed as reported previously [14]. Mice were injected intravenously with 200 μl of 0.5% Evans blue solution containing 500 μg of Ova. Anaphylactic shock was measured by 3 criteria. Ova-induced mast cell degranulation leads to vascular leakage, resulting in extravasation of both fluid and Evans blue dye into the skin. This could be detected by diffuse blueing of the skin. Anaphylaxis also induced neurological symptoms, in terms of changes in spontaneous activity and responsiveness to external stimuli (tables 2, 3). The severity of anaphylactic symptoms was

Table 3. The severity of anaphylaxis increased with the number of antigen applications and correlated with IgE levels

Shock criteria	Time min	Sensitisation strategy ¹				
		1 $\times$	1 $\times$	2 $\times$	6 $\times$	20 $\times$
Intensity of blueing ²	15	0 ³	1	1	1	1
Spontaneous activity ⁴	2	0	0	0.25	0.25	0.25
	4	0	1	1.25	0.5	0.75
	6	0	1	1.5	1.25	1.5
	8	0	1	1.5	1	1.5
	10	0	1	1.5	1.25	2
	15	0	1	1.5	2	2
Responsiveness to external stimuli ⁵	2	0	0	0	0	0
	4	0	0.75	0.5	0.25	0.5
	6	0	1	1	1	1
	8	0	1	1.25	1	1
	10	0	1	1.25	1	1.5
	15	0	1	1.5	1.5	2

¹ The total amount of antigen (Ova) for the various sensitisation strategies was 0, 100, 600, 300 and 300 μg , respectively.² Scoring scale: no = 0; blue = 1.³ Mean score rate in one group (n = 5).⁴ Scoring scale: running around = 0; sitting passively = 1; lying = 2.⁵ Scoring scale: running away upon touching = 0; slight reaction = 1; no reaction = 2.

scored by two independent observers, who were unaware of the sensitisation status of each animal.

Bronchoalveolar Lavage

On days 42 and 43 after the first immunisation, mice were challenged intranasally with 50 µg of Ova. On day 44, the mice were killed, the trachea was cannulated and bronchoalveolar lavage (BAL) was performed by flushing the lung and airways 3 times with 1 ml of PBS. BAL cells were counted, spun onto glass slides using a cytopspin and stained with Diff-Quik (Dade, Marburg, Germany) according to the manufacturer's instructions. Total cell counts and the number of eosinophils were determined microscopically using standard cytological criteria.

Histological Analysis

On days 42, 43 and 44 after the first immunisation, mice were challenged intranasally with 50 µg of Ova. Then the mice were killed, and the lung tissues were fixed in 10% phosphate-buffered formalin for 24 h and embedded in paraffin wax. Sections (2–3 µm) were cut and stained with haematoxylin and eosin or periodic acid-Schiff. The stained sections were visualised by light microscopy using standard histological criteria. The density of goblet cells was scored in the periodic acid-Schiff stain. The inflammatory infiltrate was examined in the haematoxylin and eosin stain.

Statistical Analysis

Statistical significance was analysed by the Mann-Whitney test. Values of $p < 0.05$ were considered statistically significant.

Results

Specific Humoral Immune Response to Ova After Intranasal, Epicutaneous and Intraperitoneal Sensitisation

We compared the extent to which the route, amount and frequency of antigen application influenced IgE and IgG1 production in comparison to IgG2a and IgG2b levels. BALB/c mice were immunised by intraperitoneal, epicutaneous and intranasal antigen exposure with various amounts of Ova. Sensitisation was performed as indicated in table 1. The mice were bled every week until day 42 after the first sensitisation and Ova-specific antibodies were measured by ELISA. Antibody titres were expressed as reciprocals of the serum dilutions which were 1.5 times greater than the background OD₄₀₅ of non-immunised mice.

Variation of the route of antigen application showed no significant difference ($p > 0.05$) in IgE levels between intranasally and epicutaneously sensitised mice. In contrast, intranasal and also epicutaneous Ova application were significantly ($p < 0.000001$) more efficient for the induction of specific IgE antibodies than the intraperitoneal route when antigen amount and immunisation frequency were kept constant (fig. 1).

The variation of antigen amount and immunisation frequency showed that intraperitoneal sensitisation led to detectable IgE synthesis starting after 1 single intraperitoneal injection of 100 µg of Ova; 10 µg Ova did not lead to sufficient immunisation. As expected, specific IgE levels increased with 2 intraperitoneal injections, reaching a cumulative dose of 600 µg of Ova. Interestingly, even higher IgE levels were found after 6 intraperitoneal injections, but a lower cumulative dose of 300 µg of Ova. The strongest IgE synthesis could be induced by applying a total of 300 µg of Ova with 20 intraperitoneal injections, indicating that IgE synthesis increases with the number of boosts but not with increasing antigen amount. The comparison of IgE levels after variation of antigen amount and immunisation frequency in intraperitoneally sensitised mice showed differences which were all highly significant ($p < 0.00001$) (fig. 1).

Repeated sensitisation resulted in steadily increasing IgE titres from day 0 to 42 and differed from strategies with single or double injections, where IgE levels showed peak doses and dropped to lower levels until day 42. Even considering that peak doses in these groups were reached at day 21 or day 35, peak IgE titres in these groups were lower than in the groups with repetitive boosts (data not shown). Specific IgG1 and IgG2b antibodies correlated in most cases with the IgE titres, except in the epicutaneous group, where IgE was selectively induced to a greater extent than IgG1 and IgG2b. The IgG2a subtype was induced at very low levels and was similar in all sensitisation protocols (fig. 1).

Intranasal Sensitisation without Adjuvant Leads to Local Airway Eosinophilia and Allergic Lung Pathology

We also wanted to determine whether repeated intranasal Ova boosts without adjuvant or previous intraperitoneal priming could induce allergic airway pathology after Ova challenge. Mice were sensitised as described in table 1, BAL was performed and the total amount of eosinophils was determined in the lavage fluid at different time points after sensitisation.

As shown in figure 2, local airway eosinophilia could be induced after 2 weeks of intranasal sensitisation and increased with every boost within 4 weeks. The histological examination of lung tissue of sensitised mice revealed a massive inflammatory infiltrate dominated by eosinophils (fig. 3b, d) and extensive goblet cell metaplasia in the bronchial epithelium (fig. 4b) in comparison to PBS-treated mice (fig. 3a, c, 4a).

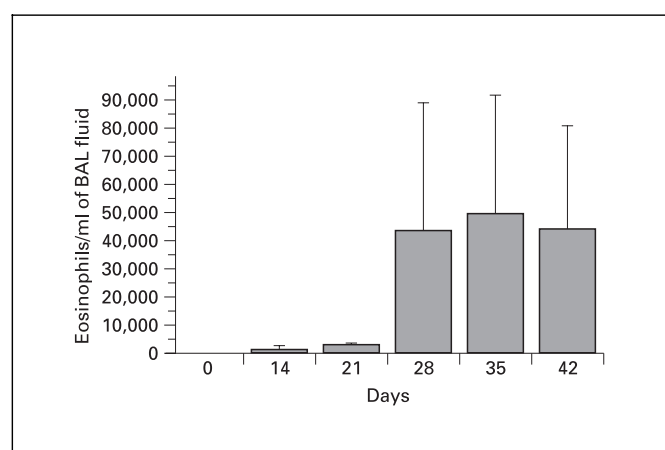
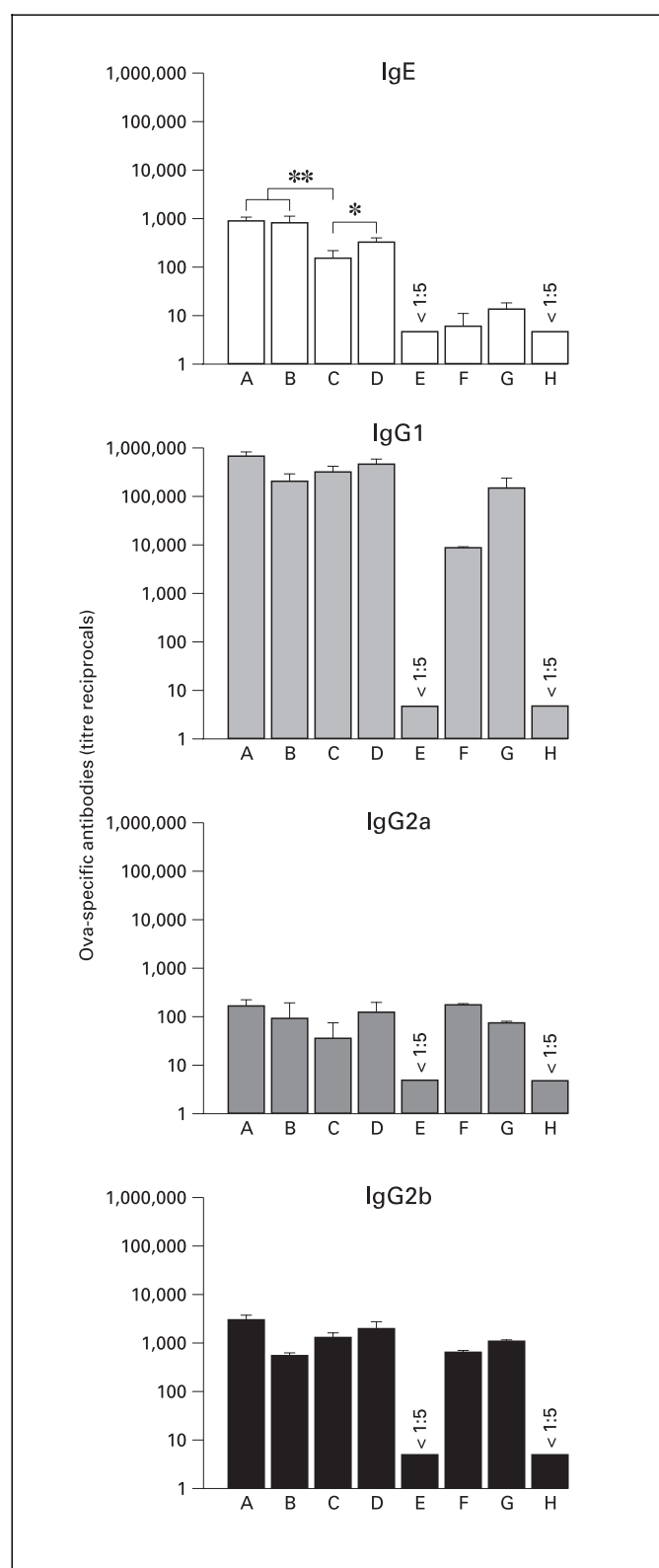


Fig. 2. Intranasal sensitisation without adjuvant leads to local airway eosinophilia. Mice were sensitised with Ova intranasally every week. BAL was performed and the total amount of eosinophils was determined in the lavage fluid at different time points after sensitisation.

Intranasal, Intraperitoneal and Epicutaneous Sensitisation Lead to Systemic Anaphylaxis

We studied whether the IgE levels in the various sensitisation protocols were high enough for the induction of systemic anaphylactic symptoms, which were measured 15 min after Ova challenge 7 weeks after the first immunisation. Interestingly, anaphylactic symptoms could be induced in all mice independently of the route of antigen application (table 2). We also compared the severity of anaphylactic symptoms every 2 min in the intraperitoneal sensitisation protocols. Table 3 shows that shock symptoms appeared earlier and the severity of shock increased with the number of antigen applications and therefore correlated well with increasing specific IgE levels.

Fig. 1. Serum levels of Ova-specific antibodies. Mice were sensitised as indicated in table 1. Ova-specific IgE, IgG1, IgG2a and IgG2b were measured by ELISA on day 42. Error bars indicate the standard deviation of mean antibody titres under each condition. Statistical significance was analysed by the Mann-Whitney test. * $p < 0.00001$, ** $p < 0.000001$. A = 6 × 50 µg of intranasal Ova (n = 15); B = 6 × 50 µg of epicutaneous Ova (n = 7); C = 6 × 50 µg of intraperitoneal Ova (n = 10); D = 20 × 15 µg of intraperitoneal Ova (n = 10); E = 1 × 10 µg of intraperitoneal Ova (n = 5); F = 1 × 100 µg of intraperitoneal Ova (n = 6); G = 1 × 100/1 × 500 µg of intraperitoneal Ova (n = 6); H = no sensitisation (n = 5).

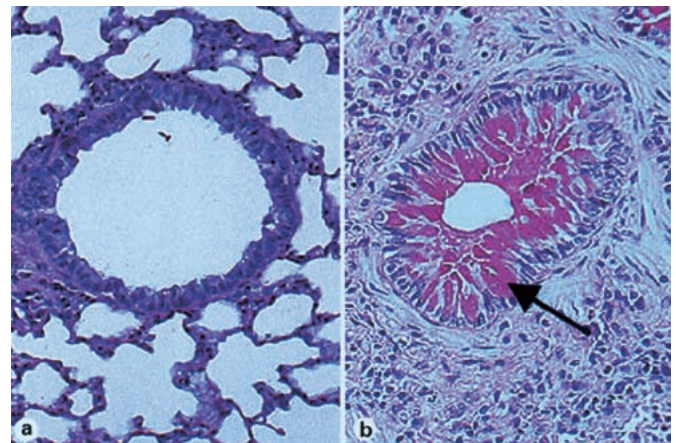
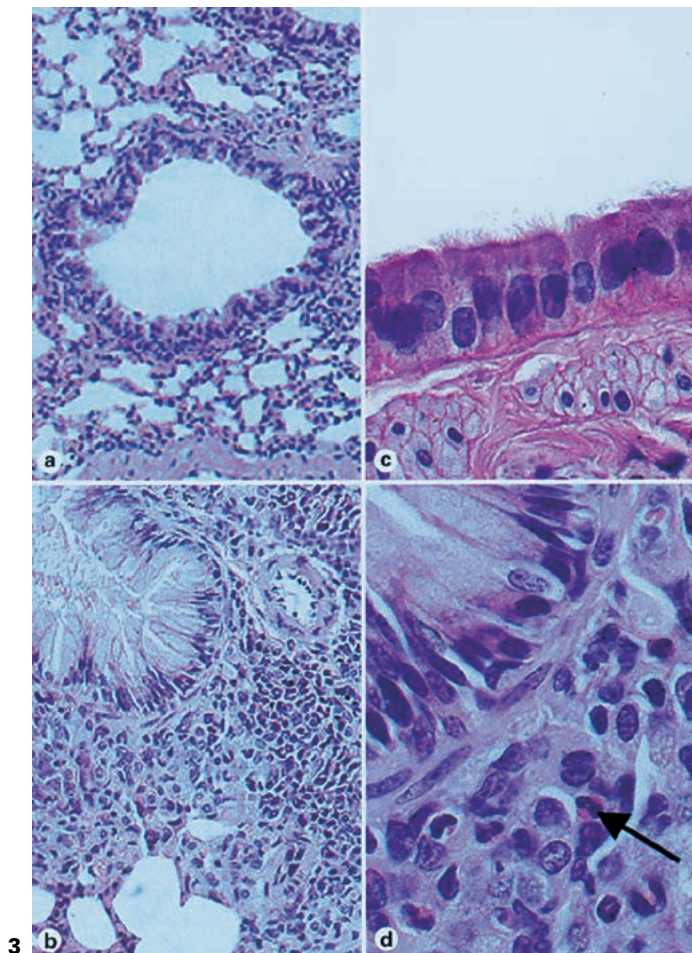


Fig. 3a–d. Intranasal sensitisation leads to inflammatory infiltrates dominated by eosinophils in the lung tissue. Mice were sensitised with Ova intranasally as described above (**b**, original magnification $\times 84$; **d**, original magnification $\times 340$; arrow = eosinophils) or immunised with PBS (**a**, original magnification $\times 84$; **c**, original magnification $\times 340$). After 6 weeks, lung tissues were fixed in formalin and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin.

Fig. 4a, b. Intranasal sensitisation leads to goblet cell metaplasia in the lung tissue. Mice were sensitised with Ova (**b**, original magnification $\times 80$; arrow = mucin granula in the goblet cells) or PBS (**a**, original magnification $\times 80$) and after 6 weeks, lung tissues were processed as described above. Sections were stained with periodic acid-Schiff.

Discussion

In this study, we compared for the first time the effects of different routes of antigen application on IgE synthesis in mice. Various routes of protein antigen application without adjuvant have already been reported to induce predominant Th2-type responses with high IgE titres, but they were not compared directly. Renz et al. [15] reported that aerosolised Ova induced increased IgE production in BALB/c mice. Wang et al. [16] showed that epicutaneous exposure to Ova induced predominant Th2-like responses with IgE production in BALB/c mice and Ito et al. [17] demonstrated that the oral route of antigen application by feeding casein also induced a predominant Th2 response with high IgE production in CBA/J mice.

We have shown that the most efficient IgE production is achieved by antigen application via the airways and skin. We also demonstrated that exclusive allergen exposure via the airways by intranasal application without pre-

vious intraperitoneal priming induces local allergic airway eosinophilia and goblet cell metaplasia. Renz et al. [15] demonstrated previously that aerosolised Ova exposure causes high IgE production and leads to increased airway hyperresponsiveness. However, allergic airway inflammation could not be induced in that study [15]. This lack of airway inflammation might be a dose-dependent effect due to differences in the application protocol of Ova. In contrast to our study, Ova was applied as an aerosol in a test chamber, where a large portion of Ova might not be inhaled or even swallowed, resulting in a very low antigen amount in the airway mucosa.

In contrast to our study, specific IgE synthesis has also been found to be suppressed with subsequent tolerance induction after aerosolised or intranasal antigen exposure in various studies [18–20]. Holt et al. [19] were able to demonstrate that aerosolised or intranasal Ova application elicited transient IgE responses. When intranasally sensitised mice were challenged intraperitoneally with

Ova, their subsequent IgE responses were markedly suppressed [19]. These differences to our findings may either be dose dependent as discussed above, or due to oral tolerance induction. In our study, mice were anaesthetised for intranasal administration of Ova in order to provide deep inhalation of the Ova solution. Using this method, most of the applied antigen should reach the bronchial mucosa. We also tried a protocol where the Ova solution was administered intranasally without anaesthesia and no detectable Ova-specific humoral immune response could be measured (data not shown). In this case, mice could be observed swallowing the solution and most of the allergen probably reached the gut. Holt et al. [19] were also able to demonstrate by radiotracer studies in their experiments that a large portion of Ova administered intranasally became associated with the gut. Therefore, the observed suppression of IgE synthesis in these experiments may not only be due to nasal but also due to oral tolerance induction. Interestingly, Wolvers et al. [20] were able to clarify a mechanism of how intranasal tolerance might be induced by demonstrating that immunological tolerance is determined by characteristics of the draining lymph nodes.

The strong induction of allergen-specific IgE after intranasal sensitisation in our study was probably due to specific features of the pulmonary cellular microenvironment. Antigen presentation in the lung may favour Th2 responses and IgE production in the bronchial mucosa, since dendritic cells which are potent APCs are present in the nasal mucosa and also in the lung tissue. These cells are increased in patients with allergic rhinitis during allergen provocation [21, 22]. Moreover, pulmonary dendritic cells may also be modulated by the local cellular and cytokine milieu in a way that promotes IgE production [7]. This hypothesis is supported by the finding that in humans, dendritic cell changes in terms of quantity and phenotype are indeed found in atopic individuals in a way that favours type 2 responses [23, 24].

Epicutaneous sensitisation also resulted in high-level IgE synthesis. In agreement with our finding, Wang et al. [16] demonstrated previously that the epicutaneous route of antigen application resulted in a dominant Th2-like immune response with high levels of IgE. In the skin, dendritic cells also act as antigen presenters. In addition, mast cells are widely distributed in the dermis and act as sentinels at sites of initial antigen exposure. They can produce IL-4 and IL-13, which may maintain a Th2 shift and amplify an allergic response in states of repetitive low-dose allergen exposure [25]. In agreement with our data, Wang et al. [16] found that repetitive epicutaneous boosts induced steadily increasing IgE synthesis. Wang et al. [16]

proposed that immune responses induced by an epicutaneous route differ from responses in the lung or the gastrointestinal tract, since previous reports showed that oral or inhaled antigen presentation induces transient IgE production, which is subsequently suppressed [26, 27]. In our study, steadily increasing IgE appeared with increasing numbers of boosts independently of the route of antigen exposure, suggesting that this effect is induced by increasing frequency of antigen application rather than being due to skin-specific phenomena [16].

The intraperitoneal strategy clearly showed that IgE and IgG1 synthesis was enhanced with increasing frequency of application but not with increasing amounts of antigen. This is supported by a study from Arps et al. [28], in which low levels of antigen predominantly induced high IgE titres in CBA/J mice sensitised to keyhole limpet hemocyanin absorbed to alum, whereas high antigen doses promoted the production of IgG2a antibodies. However, whether the reciprocal regulation of IgE and IgG1 versus IgG2a isotype production is due to differential activation of Th1 versus Th2 cells is controversial in the literature. Arps et al. [28] showed that reciprocal regulation of antibody production was not due to differential activation of Th1 and Th2 populations, because Th2 cell populations were induced with both high and low doses of antigen. This was supported by an *in vitro* study of Hosken et al. [29], who demonstrated that very high and low doses of peptide antigen induced Th2-type responses with IL-4 secretion in T cell cultures, whereas mid-range antigen levels induced Th1-type responses and IFN- γ production. In contrast, Guery et al. [30] found that protein antigens, which were continuously applied subcutaneously with a mini osmotic pump, induced optimal priming of Th2 cells with low doses of antigen rather than with high doses.

All data presented in this study were obtained by sensitisation protocols without the use of an adjuvant. However, we also integrated two groups of mice with a single or double intraperitoneal injection of 10 μ g of Ova with aluminum hydroxide as adjuvant (data not shown). The use of an adjuvant may enhance an antibody response but also induces non-specific T cell inflammatory responses as well as several cellular functions [31]. As demonstrated before, aluminum hydroxide as an adjuvant allowed efficient IgE synthesis also in our study [32]. The threshold of induction of a humoral immune response was lowered, since the use of an adjuvant already induced an Ova-specific immune response after a single injection of 10 μ g of Ova. As expected, IgE levels increased after a boost into the range of those mice which had been sensitised with $6 \times 50 \mu$ g of

Ova intraperitoneally without adjuvant, but the Ova-specific antibody synthesis was much more polarised towards IgG2a and IgG2b using this protocol (data not shown).

Although there were large differences in IgE production between the various sensitisation strategies of intranasal, epicutaneous and intraperitoneal antigen application, all resulted in clinically relevant IgE and IgG1 synthesis. The intensity of the anaphylactic symptoms varied, but all antibody levels were high enough for the induction of systemic allergic symptoms in terms of anaphylactic shock. This is probably due to the genetic background of the BALB/c mouse strain, which has been characterised as a high IgE responder, and also to the use of Ova, which is a well-established IgE-inducing antigen in this system [29].

Taken together, our data reflect the situation in humans, where IgE induction and allergic sensitisation can occur after antigen delivery via various routes. Sensitisation via the airway or gut mucosa results in inhalation or food allergies. Antigen exposure on the skin may lead, for example, to latex allergy, and systemic sensitisation re-

sults in hymenoptera or drug allergy, for example. This animal model, however, demonstrates that antigen application via the airways or skin induces IgE synthesis more efficiently than via the intraperitoneal route. Few exposures with high-dose antigen are less efficient than multiple exposures with low doses. Our finding that both the route and the frequency of antigen application strongly influence IgE synthesis may help to understand how environmental antigens lead to allergic sensitisation. This knowledge may be of specific importance for optimising protocols of allergen-specific immunotherapy.

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