THE effects of different sensitization and allergen provocation regimens on the development of allergen-induced bronchial hyperreactivity (BHR) to histamine were investigated in conscious, unrestrained guinea-pigs. Similar early and late phase asthmatic reactions, BHR for inhaled histamine after the early (6 h) as well as after the late reaction (24 h), and airway inflammation were observed after a single allergen provocation in animals sensitized to produce mainly IgG or IgE antibodies, respectively. Repeating the allergen provocation in the IgE-sensitized animals after 7 days, using identical provocation conditions, resulted in a similar development of BHR to histamine inhalation. Repetition of the allergen provocation during 4 subsequent days resulted in a decreased development of BHR after each provocation, despite a significant increase in the allergen provocation dose necessary to obtain similar airway obstruction. The number of inflammatory cells in the bronchoalveolar lavage was not significantly changed after repeated provocation, when compared with a single allergen provocation. Finally, we investigated allergen-induced bronchial hyperreactivity by repetition of the sensitization procedure at day 7 and 14 (booster), followed by repeated allergen provocation twice a week for 5 weeks. Surprisingly, no BHR to histamine could be observed after either provocation, while the number of inflammatory cells in the bronchoalveolar lavage fluid after 5 weeks was enhanced compared with controls. These data indicate that both IgE and IgG sensitized guinea-pigs may develop bronchial hyperreactivity after a single allergen provocation. Repeated allergen exposure of IgE sensitized animals causes a gradual fading of the induced hyperreactivity despite the on-going presence of inflammatory cells in the airways, indicating a mechanism of reduced cellular activation.

Key words: Allergic asthma, Bronchial hyperreactivity, Guinea-pigs, IgE, IgG, Inflammation, Sensitization

Influence of sensitization and allergen provocation procedures on the development of allergen-induced bronchial hyperreactivity in conscious, unrestrained guinea-pigs

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Introduction

Allergen-induced early (EAR) and late (LAR) phase asthmatic reactions, bronchial hyperreactivity (BHR), and airway inflammation are common characteristics of allergic asthma. Bronchial reactivity increases upon exposure to airborne allergens and appears to be related to the development of a LAR, since enhanced reactivity to inhaled histamine and methacholine was observed in asthmatic patients responding to allergen provocation with an EAR as well as a LAR (dual responders), while no increase was found in patients who developed an EAR only.^{1–3} It was also observed that bronchial reactivity was already increased before the LAR was clinically manifest, suggesting that this early developed BHR may contribute to the severity of the LAR.³ The development of the LAR and the allergen-induced increase in bronchial reactivity are associated with an influx of inflammatory cells into the airways,^{4,5} indicating that

airway inflammation may be involved in these processes.

The class and concentration of circulating reagenic antibodies may determine to a large extent the development of clinical symptoms of allergic asthma. Cutaneous late phase reactions are IgE-dependent and can be transferred passively; the ability to perform these passive transfers is eliminated by removal of the IgE antibodies from the serum and restored by administration of the IgE fraction.^{6,7} Evidence that LARs are also IgE-dependent comes from the observation that they can be produced by inhalation of anti-IgE antibodies.⁸

Experimentally induced IgE and IgG reagenic antibody formation in guinea-pigs greatly depends on the sensitization and allergen provocation procedures applied.^{9,10} Addition of Al(OH)₃ to the sensitization solution facilitates the production of IgE antibodies after parental administration of this solu-

tion, whereas in the absence of adjuvant or in the presence of Freund's Complete Adjuvant mainly IgG antibodies are produced.^{9,10} Tracheal instillation of an immobilized allergen (ovalbumin coupled to Sepharose) caused a dual asthmatic reaction in guinea-pigs sensitized to IgE, whereas in guinea-pigs sensitized to IgG, only an EAR was observed.¹¹ The influence of IgE and IgG sensitization procedures on the development of early and late asthmatic reactions, BHR and airway inflammation after inhalational challenge with aerosolized allergen is, however, unclear.

Therefore, using a new model of chronically instrumented, conscious and unrestrained guinea-pigs,¹² we investigated these responses in two groups of guinea-pigs that were sensitized to produce mainly IgG or IgE antibodies to ovalbumin, respectively.

Similar to the allergic responses in human asthmatics, we have shown recently that the IgE-sensitized guinea-pigs develop dual asthmatic reactions as well as BHR to histamine and airway inflammation after these reactions following a single allergen challenge with ovalbumin aerosol.¹³ The effects of more prolonged, repeated exposures to allergen on airway reactivity in this model are still unclear. Such exposures could lead to enhancement of inflammatory responses in the airways and therefore to a greater degree of BHR than that observed after a single exposure. Since chronic natural inhalational exposure of asthmatic patients to allergen may indeed be relevant to the development of BHR in these patients,¹⁴ we subsequently tried to enhance allergen-induced development of BHR to inhaled histamine by increasing the frequency of allergen provocations. In a final protocol, we tried to optimize the possible development of BHR by repeating the sensitization procedure (booster) in combination with a further increase in the number of allergen provocations.

Methods

Sensitization and challenge procedures: Specific pathogen-free guinea-pigs (Charles River SAVO, Kiszlegg, Germany), weighing approximately 300 g, were used in this study. The animals were housed in individual cages in climate-controlled animal quarters and given water and food *ad libitum*.

For the first part of this study, the animals were divided into three separate groups. The first group (control) was not sensitized. The second group (IgG) was sensitized with an emulsion of equal volumes of saline and Freund's Complete Adjuvant, containing 100 µg/ml ovalbumin. The third group (IgE) was sensitized with a suspension of 100 mg/ml Al(OH)₃ in 100 µg/ml ovalbumin in saline.

The antigen solution (0.5 ml) was injected i.p. and

another 0.5 ml was divided over seven s.c. injection sites in the proximity of lymph nodes in the paws, lumbar regions and neck. These procedures facilitate selective production of IgG and IgE antibodies, respectively, as determined by passive cutaneous anaphylaxis.¹⁰ The animals were challenged by aerosol 4–5 weeks after sensitization with either saline (control) or increasing concentrations of ovalbumin (1, 3, 5, 7, 10, 20 mg/ml) for 3 min with 10 min intervals, until signs of respiratory distress were observed.

In the second part of this study only the IgE-sensitization procedure described above was used. The sensitized animals were challenged with ovalbumin aerosol as described above either once (IgE), twice (IgE–2×) or four times (IgE–4×). The interval between subsequent allergen provocations was 7 days (IgE–2×) or 24 h (IgE–4×). In a final group (IgE–10×), the IgE sensitization described above was repeated at day 7 and 14 (boostering). These animals were challenged with allergen twice a week for 5 weeks, starting 14 days after the last sensitization.

All protocols were approved by the University of Groningen Animal Health Committee, which is responsible for the care and proper use of experimental animals.

Measurement of airway function: Airway function was assessed by P_{pl} measurement as described previously.¹² In brief, a small latex balloon, connected to a saline-filled tube, was surgically implanted inside the thoracic cavity. The cannula was driven subcutaneously and permanently attached to the neck of the animal. After connection via another fluid-filled cannula to a pressure transducer (Gould P23ID, Gould Medical BV, Bithoven, The Netherlands), P_{pl} was measured using an on-line computer system. No post-surgical inflammation was observed for at least 5 weeks after operation and baseline P_{pl} values remained stable during repeated measurements on different days.¹² Using a combination of flow measurement with a pneumotachograph, implanted inside the trachea, and pressure measurement with the pleural balloon, we showed that changes in P_{pl} are linearly related to changes in airway resistance and hence can be used as a sensitive index for histamine- and allergen-induced bronchoconstriction.¹²

Provocation procedures: For aerosol provocation, an animal cage was developed in which the guinea-pigs could move freely.¹² The volume of the cage was 9 l, which ensured a fast replacement of the air inside the cage with aerosol and vice versa. A De Vilbiss nebulizer (type 646, De Vilbiss, Somerset, PA, USA) driven by an airflow of 8 l/min, provided the aerosol required, with an output of 0.43 ml/min.

The animals were habituated to the experimental conditions on 2 separate days. Subsequently, animals were challenged with saline, histamine and

ovalbumin aerosols. All provocations were preceded by an additional adaptation period of at least 30 min, followed by two consecutive control challenges with saline.

Histamine provocations were performed with a 25 µg/ml solution of histamine in saline, followed by increasing dosage steps of 25 µg/ml. Each provocation lasted 3 min and was separated by 10 min intervals. Animals were challenged until the P_{pl} increased by more than 100% for at least 3 consecutive min. The provocation concentration causing a 100% increase in P_{pl} (PC_{100}) was derived by linear interpolation.

Experimental protocol: To establish baseline histamine PC_{100} -values, two provocation sessions were performed, separated by 3 days. Variations between the first and second PC_{100} -values were small ($3.8 \pm 2.6\%$, $n = 31$).

One day after the second histamine provocation allergen provocations were performed. At 6 h (between the EAR and LAR) and 24 h (after the LAR) following allergen exposure to IgG and IgE sensitized guinea-pigs, histamine PC_{100} -values were re-established to assess changes in airway reactivity at these time points. The second prechallenge histamine PC_{100} -value was used to calculate a ratio in PC_{100} pre/post provocation with saline or allergen. At the allergen provocation day, P_{pl} was continuously measured during the whole procedure to assess allergen-induced early and late phase asthmatic responses. Between the measurements of histamine PC_{100} at 6 h and 24 h, the animals were removed from the provocation cage and placed in a large cage, where they could move around freely and eat and drink *ad libitum*. During this transfer, the animals remained connected to the measurement system.

The effect of repeated allergen provocations on the development of BHR was investigated using IgE-sensitized guinea-pigs only. One group of animals (IgE-2×) was re-exposed to allergen 7 days after the first provocation. The other group (IgE-4×) was exposed daily for 4 subsequent days, until comparable bronchoconstriction occurred.

In the final group of animals the IgE sensitization procedure was repeated at day 7 and 14 (booster) after the initial sensitization. These animals were exposed to allergen twice a week for 5 weeks, starting at day 28. In this group airway reactivity to histamine inhalation was assessed at 24 h before and after the first allergen provocation and (once a week) 24 h after the 3rd, 5th, 7th, 9th and 10th allergen provocation.

Bronchoalveolar lavage procedure: The bronchoalveolar lavage was performed 24 h after a single allergen or saline provocation or 24 h after the last repeated allergen provocation. Animals were anaesthetized with 20 mg/ml Brietal-sodium, 35 mg/kg

ketamine hydrochloride and 6 mg/kg Rompun i.p. which ensured a fast, deep anaesthesia. The trachea was exposed and the lungs were gently lavaged via a tracheal cannula with 5 ml of sterile saline at 37°C, followed by three subsequent aliquots of 8 ml of saline. The recovered lavage samples were cooled on ice, and centrifuged at $200 \times g$ for 10 min at 4°C. The combined pellets were resuspended to a final volume of 1.0 ml in RPMI-1640 medium. Total cell numbers were counted in a Bürker-Türk chamber. For cytological examination, cytospin preparations were made in a Shandon cytospin-2 cytocentrifuge (Shandon Southern Instruments, Swickley, PA, USA) using 100 µl aliquots of the cell suspension, spinning at $300 \times g$ for 5 min. The preparations were stained with May-Grünwald and Giemsa. A cell differentiation was performed by counting 400 cells in duplicate.

Chemicals: Histamine hydrochloride, ovalbumin (grade III), and aluminum hydroxide were obtained from Sigma Chemical Co. (St Louis, MO, USA). Freund's Complete Adjuvant was purchased from Difco Laboratories (Detroit, MI, USA), Brietal-sodium (methohexital) from Eli Lilly (Amsterdam, the Netherlands), ketamine hydrochloride from Parke-Davis (Barcelona, Spain), Rompun (2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine-hydrochloride, methylparaben) from Bayer (Leverkusen, Germany), and RPMI-1640 medium from Gibco Life Technologies (Paisley, UK).

Data analysis: The magnitude of the EAR after allergen provocation was expressed as the area under the percentage change in P_{pl} -time curve (AUC) between 0 and 6 h after provocation, calculated by trapezoid integration over 5 min periods. The magnitude of the LAR was expressed as the AUC between 8 and 24 h after provocation.

Development of BHR after provocation was evaluated by Student's *t*-test for paired observations. Student's *t*-test for unpaired data was used to compare data from different groups of animals. Differences were considered to be significant when $p < 0.05$. All data are presented as means $\pm$ S.E.M.

Results

Influence of the sensitization regimen: In the first part of this study the development of early and late phase asthmatic reactions, bronchial hyperreactivity and airway inflammation induced by a single allergen provocation was investigated in guinea-pigs sensitized to produce predominantly IgG or IgE antibodies. Allergen-induced asthmatic responses are presented in Table 1. Animals from both sensitization groups responded to allergen provocation with a marked early bronchospasm (IgG, $p < 0.01$; $p < 0.001$, respectively). In most animals (four of six IgG ani-

Table 1. Airway responses of guinea-pigs after saline or allergen provocation

Sensitization and provocation procedure	n	AUC early reaction (%.5 min)	AUC late reaction (%.5 min)
Non-sensitized, saline provocation	7	160 ± 54	873 ± 412
IgG sensitized, allergen provocation	6	4990 ± 1499**	4373 ± 1118**
IgE sensitized, allergen provocation	9	3490 ± 578***	6700 ± 1092***

Definition of abbreviations: AUC = Area under % P_{pl} -time curve. Data represent mean values ± S.E.M. Statistical analysis: unpaired Student's *t*-test, compared with saline provocation; ***p* < 0.01; ****p* < 0.001.

mals and eight of nine IgE animals), the early reaction was followed by a late asthmatic reaction, which was significantly different from control in both groups (IgG, *p* < 0.01; IgE, *p* < 0.001, respectively). No significant differences in the magnitudes of the EAR and LAR were observed between the IgG and IgE animals (Table 1).

The development of BHR to histamine appeared to be equal in both sensitization regimens applied, when compared with control animals (Table 2). At 6 h after allergen provocation (i.e., between the EAR and LAR), when P_{pl} had returned to baseline values, bronchial reactivity was significantly increased in IgG (*p* < 0.05), as well as in IgE sensitized animals (*p* < 0.01). At 24 h after allergen provocation, i.e., after resolution of the LAR, bronchial reactivity was diminished, but still significantly enhanced for the IgG group (*p* < 0.05), as well as for the IgE animals (*p* < 0.01) (Table 2).

Examination of the cell content of the bronchoalveolar lavage fluid revealed that the numbers of eosinophils and neutrophils, but not of lymphocytes and macrophages, were significantly and similarly enhanced at 24 h after allergen provocation, when compared with control, for IgG and IgE sensitized animals (Fig. 1).

Influence of provocation protocol: In animals sensitized to raise IgE antibodies, the development of BHR to histamine at 6 h as well as at 24 h after each of repeated allergen provocations was investigated using different challenge protocols (Table 3). The development of BHR to histamine inhalation after the first allergen exposure in each protocol corresponded closely to that observed in the single challenged animals. Repetition of the allergen provocation 7 days later (IgE-2×), using the same allergen concentration(s) as before resulted in a comparable development of BHR (Table 3). With daily repeated allergen provocations (IgE-4×), challenging until respiratory distress was observed, the developed BHR diminished with each subsequent provocation (Table 3), despite a significant increase in allergen provocation concentration from 2.3 ± 0.4 mg/ml on the first day to 4.0 ± 1.1 mg/ml on the last day (*p* < 0.05, paired one-sided Student's *t*-test, *n* = 6).

The number of eosinophils in the bronchoalveolar lavage fluid when assessed at 24 h after the last

allergen provocation, was significantly increased compared with control animals in both groups (IgE-2×, *p* < 0.01; and IgE-4×, *p* < 0.001). The number of neutrophils tended to increase after the second provocation in the IgE-2× group (*p* = 0.06), while only a significant increase was found after the fourth provocation in the IgE-4× group (*p* = 0.02). In these groups the numbers of lymphocytes and macrophages as well as the total number of cells were unchanged compared with control animals (Fig. 2). All cell numbers in the repeated challenge protocols were not significantly different from those observed in single challenged IgE guinea-pigs.

In an attempt to optimize the development of BHR, a final group of IgE sensitized animals was re-sensitized twice and subsequently exposed to allergen twice a week for 5 weeks. The allergen concentration, necessary to obtain an allergic obstruction, increased from 1.0 ± 0.0 mg/ml on the first day to 8.7 ± 5.7 mg/ml on the last day (N.S., *n* = 3). However, a significant increase in bronchial reactivity to inhaled histamine was not observed (Fig. 3). Surprisingly, and in contrast to the former protocols, BHR had not developed at 24 h after the first provocation. The number of eosinophils, neutrophils and lymphocytes in the bronchoalveolar lavage fluid at 24 h after the tenth allergen provocation tended to be diminished compared with those observed at 24 h after a single provocation. Compared with control animals, the numbers of eosinophils (*p* < 0.001) and neutrophils (*p* < 0.01) were still significantly enhanced, however (Fig. 2).

Discussion

The choice of adjuvant in the sensitization solution determines the distribution of reagenic antibodies produced. Addition of FCA thus resulted in production of almost exclusively (99%) IgG antibodies, whereas in animals treated with Al(OH)₃ 44% of the antibodies were of the IgE class.¹⁰ Despite the apparent relationship between the level of circulating IgE and the severity of the asthmatic symptoms in patients,¹⁵ a clear difference in the severity of the allergen-induced late asthmatic reaction between IgG and IgE sensitized guinea-pigs was not observed

Table 2. Bronchial reactivity to histamine in guinea-pigs after saline or allergen provocation.

Sensitization and provocation procedure	n	Ratio PC ₁₀₀ (pre/post-challenge) at different times after provocation	
		6 h	24 h
Non-sensitized, saline provocation	7	1.06 ± 0.08	1.06 ± 0.09
IgG sensitized, allergen provocation	6	2.96 ± 0.69*	1.69 ± 0.24*
IgE sensitized, allergen provocation	9	2.93 ± 0.50**	1.56 ± 0.12**

Definition of abbreviations: PC₁₀₀ = provocation concentration of histamine causing 100% increase in pleural pressure. Data represent mean values ± S.E.M. Statistical analysis: unpaired Student's *t*-test, compared with saline provocation; **p* < 0.05; ***p* < 0.01.

FIG. 1. Cell content of bronchoalveolar lavage fluid, obtained at 24 h after saline (□), or allergen provocation of IgE (▨) or IgG (▩) sensitized guinea-pigs. Abbreviations: eo = eosinophils, neutro = neutrophils, lympho = lymphocytes, and macro = alveolar macrophages. Data are presented as mean ± S.E.M. Statistical analysis: unpaired Student's *t*-test, compared with saline challenge, ***p* < 0.01; ****p* < 0.001).

in this study. This is in contrast with the studies performed by Wieslander and colleagues,¹¹ who reported that the magnitude of the LAR was determined by the IgE level. On the other hand, Hutson and coworkers¹⁶ observed a LAR in all guinea-pigs, sensitized to produce IgG antibodies, while Johns *et al.*¹⁷ observed an LAR in only 28% of the animals using the same sensitization and allergen provocation protocols. In the present study, the development

of allergen-induced late asthmatic reactions and BHR to histamine, as well as airway inflammation appeared to be comparable between the IgG and IgE sensitization regimens. Bronchial reactivity to histamine was already increased at 6 h after allergen provocation, which might have contributed to the magnitude of the LAR.¹³ However, BHR after the LAR was lower than BHR preceding the LAR, indicating that the LAR *per se* did not substantially contribute to the development of BHR as is currently believed.^{2,3}

In the second part of this study attempts were made to increase the development of BHR. Since in asthmatic patients the role of IgE has been well established,¹⁵ the IgE sensitization procedure was chosen in order to investigate the influence of repeated allergen exposure. Repetition of allergen inhalation 1 week after the first provocation, using identical provocation conditions, did not result in increased BHR, but similar levels were obtained after the EAR as well as after the LAR. This is in agreement with earlier studies by Cockcroft and colleagues^{18,19} who described reproducible EARs and LARs and allergen-induced BHR in 22 asthmatics when the interval between two allergen provocations was longer than 5 days. However, daily repetition of allergen exposure for up to 4 days tended to decrease the development of BHR, despite a small but significant increase in the allergen concentration

Table 3. Bronchial reactivity to histamine in IgE sensitized guinea-pigs after saline or allergen provocation

Provocation procedure	n	Ratio PC ₁₀₀ (pre/post-challenge) at different times after provocation	
		6 h	24 h
Saline provocation	8	1.01 ± 0.08	0.93 ± 0.07
Allergen provocation	9	2.93 ± 0.50**	1.56 ± 0.12**
Repeated allergen provocation			
1st challenge (day 1)	7	2.36 ± 0.22***	2.06 ± 0.21**
2nd challenge (day 8)	7	2.74 ± 0.43**	1.56 ± 0.18*
Repeated allergen provocation			
1st challenge (day 1)	6	4.20 ± 1.12*	1.64 ± 0.30*
2nd challenge (day 2)	6	1.96 ± 0.38*	1.49 ± 0.29
3rd challenge (day 3)	6	1.82 ± 0.35*	1.40 ± 0.25
4th challenge (day 4)	6	1.34 ± 0.09*	1.29 ± 0.12

Definition of abbreviations: PC₁₀₀ = provocation concentration of histamine causing 100% increase in pleural pressure. Data represent mean values ± S.E.M. Statistical analysis: unpaired Student's *t*-test, compared with saline provocation; **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

FIG. 2. Cell content of bronchoalveolar lavage fluid, obtained at 24 h after saline (□), or the final allergen provocation of IgE sensitized guinea-pigs, exposed once (▨, IgE group), twice (▧, IgE-2× group), four times (▩, IgE-4× group), or ten times (■, IgE-10× group). For protocols see text. Abbreviations: eo = eosinophils, neutro = neutrophils, lympho = lymphocytes, and macro = alveolar macrophages. Data are presented as mean ± S.E.M. Statistical analysis: unpaired Student's *t*-test, compared with saline challenge, **p* < 0.05; ***p* < 0.01; ****p* < 0.001).

necessary to obtain an allergen-induced bronchial obstruction.

Since mast cells are involved in the initial processes after allergen provocation, the increasing allergen concentrations may suggest exhaustion of the mast cell population involved when the interval between allergen provocations is short. Indeed, there is evidence that mast cells are not replenished after degranulation.²⁰ In a recent study, we have shown that the development of BHR after the EAR as well as the LAR in single allergen-challenged guinea-pigs

FIG. 3. Bronchial reactivity to inhaled histamine in IgE sensitized guinea-pigs, boosted twice at day 7 and 14. Allergen exposures were performed twice a week for 5 weeks. Bronchial reactivity was determined once a week, at 24 h after each odd allergen provocation as well as at 24 h after the final allergen provocation. Statistical analysis: paired Student's *t*-test, compared with prechallenge PC₁₀₀. No statistically significant differences were observed.

is strongly dependent upon the initial degree of airways obstruction during the EAR,¹³ which is predominantly caused by the release of histamine from mast cells.²¹ In addition, we have shown that inhalation of the H₁ histamine receptor antagonist mepyramine (1 mg/ml, 10 min) 1 h before allergen provocation prevented the development of both early and late BHR to both histamine and methacholine,²² indicating that histamine contributes to the development of allergen-induced BHR and that exhaustion of participating mast cells could thus be involved in the diminished development of BHR after repetitive allergen provocations. In a final attempt to increase the severity of allergen-induced BHR IgE sensitized animals were boosted at day 7 and 14, followed by repetitive allergen provocations twice a week for 5 weeks. Bronchial reactivity was measured every week and did not increase. The failure of booster sensitization to induce BHR after the first allergen provocation was surprising, because using a booster has been claimed to increase BHR,^{23,24} as well as IgE titres and the severity of the LAR.²⁵ It is possible that the 14 days between the last sensitization and the first allergen provocation was too short to enable sufficient IgE production. This is, however, unlikely since sensitivity to allergen is correlated with IgE titres,¹⁵ and each animal in this group responded to the lowest allergen concentration applied.

Investigation of airway inflammation by assessment of inflammatory cell numbers in the bronchoalveolar lavage fluid, revealed that repetitive allergen provocations did not further enhance the numbers of eosinophils and neutrophils, compared with a single allergen provocation, while the number of lymphocytes and macrophages remained unchanged.

There is considerable evidence to support the hypothesis that eosinophils are involved in the pathogenesis of asthma. The release of mediators from activated eosinophils may, for example, cause epithelial damage,²⁶ mucosal oedema and reduced mucus clearance,²⁷ dysfunction of the autoinhibitory muscarinic M₂ receptor,²⁸ and possibly also a reduced β-adrenoceptor sensitivity of the airway smooth muscle.^{29,30} In this context, the observation that BHR did not increase after repeated allergen exposure in this model is not surprising, since eosinophilic infiltration also failed to increase. Nevertheless, the (continuous) presence of inflammatory cells in the airways and the reduction or even absence of BHR in the repeated provocation protocols suggest that airway inflammation is not always associated with BHR, as has also been suggested by other studies (for view see Reference 31). A dissociation between the development of inflammatory cell infiltration in the air-

ways and the absence of BHR after repeated allergen challenge was recently also observed in other studies of ovalbumin sensitized guinea-pigs³² and rats.^{33,34} In addition, in mild asthmatics it was recently shown that two repeated allergen provocations within 48 h did not lead to enhanced early and late obstructive reactions and BHR after the second challenge.³⁵

The results from the present and the above-mentioned studies may point to a compensatory mechanism inhibiting the development of increased BHR after repeated allergen exposure by inducing a reduced activation state of the inflammatory cells present in the airways. This could possibly result from a reduced release of histamine as discussed above. In addition, activation of eosinophils and subsequent development of BHR appears to depend on the production of a cytokine profile that is characteristic for T helper lymphocytes of the Th₂ phenotype.³⁶ The latter cells secrete the interleukins IL-3, IL-4 and IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) and increased mRNA expression of these cytokines has been found in BAL cells and bronchial biopsies from allergic asthmatics.^{37,38} IL-5, together with IL-3 and GM-CSF, enhances infiltration, activation and survival of eosinophils, while IL-4 promotes the switching of B cell isotypes to production of IgE. On the other hand, proliferation of Th₂ cells as well as the B cell isotype switch are inhibited by interferon- γ (IFN- γ), which, together with IL-2, is particularly produced by Th₁-type lymphocytes.³⁶ Recently, it has been demonstrated that immunotherapy by repeated intradermal allergen challenge increased the expression of mRNA for both IFN- γ and IL-2 in skin biopsies of patients with hay fever, indicating that a shift to a Th₁ cell-specific cytokine profile may be involved in the antigen-induced suppression of the allergic immune response.³⁹ This was also indicated by the recent observation in mice that repeated inhalation of ovalbumin causes activation of antigen-specific CD8⁺ $\gamma\delta$ ⁺ T cells, which may suppress Th₂ cell-mediated specific IgE production as well IgG₁ synthesis and concomitantly enhance IgG_{2a} secretion, presumably by the release of high levels of IFN- γ by these cells.⁴⁰

In conclusion, the results described in this paper indicate that an early increase in bronchial reactivity towards inhaled histamine in association with the development of early and late phase asthmatic reactions and airway inflammation can be observed after a single allergen provocation in guinea-pigs, irrespective of whether mainly IgG or IgE antibodies had been raised. The inability to enhance the severity or duration of allergen-induced BHR by increasing the frequency of the sensitization and provocation procedures indicates that additional treatment, for example the use of *Bordetella pertussis* vaccine as an adjuvant,⁴¹ is necessary for this purpose.

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