

Dissociation of airway responsiveness and bronchoalveolar lavage (BAL) cell composition in sensitized guinea-pigs after daily inhalation of ovalbumin

H. O. HEUER, B. WENZ*, H. M. JENNEWEIN and K. URICH*

Department of Pharmacology, Boehringer Ingelheim KG, Ingelheim/Rhein and *Faculty of Biology, Johannes Gutenberg University, Mainz, Germany

Summary

The association between inflammatory cell influx, cell activation status and change of airway responsiveness to acetylcholine (ACh) after daily inhalation of ovalbumin (OA) in sensitized guinea-pigs was investigated. Starting 3 weeks after sensitization (OA at 50 mg/kg s.c. + i.p.) guinea-pigs were exposed daily to 2% OA (10 min; under cover of 0.5 mg/kg mepyramine i.p. 15 min before OA) for 2 weeks. Concentration-response curves (CRCs) for inhaled ACh were performed 24 h after the last OA-challenge and 24 h after another single OA-inhalation 1 week later. CRCs for inhaled ACh were neither affected 24 h after the last OA challenge (daily for two weeks) nor 24 h after another OA-inhalation one week later. In contrast, bronchoalveolar lavage (BAL) from repeatedly OA-sensitized/-challenged guinea-pigs immediately after the last CRC showed a significant increase of total cell count by about tenfold and increases in eosinophils by about 20-fold, neutrophils by 30-fold, macrophages by about fivefold and lymphocytes by about tenfold ($P < 0.05$, multiple Wilcoxon-test). In contrast, markers of cell activation (EPO, MPO) were significantly decreased ($P < 0.05$). Methylprednisolone almost completely prevented these changes in increased cell numbers and decreased cell activation (vs OA contr., $P < 0.05$). The lack of increased airway hyperresponsiveness despite a massive inflammatory cell influx suggests other factors controlling airway responsiveness than inflammation.

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Introduction

The mechanisms of increased airway responsiveness, a key feature of bronchial asthma, are still unclear. Sustained inflammatory cell influx into lung tissue and bronchoalveolar lavage (BAL) have been described to be chronologically associated with the occurrence of the late-phase reaction and airway hyperresponsiveness in bronchial asthma and animal models thereof [1–4]. However, it is still under discussion, if and which inflammatory cells contribute to the development of airway hyperresponsiveness [1,4].

Recently Ladenius *et al.* [5] described a model in the

guinea-pig achieving long-lasting and pronounced airway hyperresponsiveness after 10 day inhalation of specific antigen to sensitized guinea-pigs.

The main aim of this study was to reproduce the airway hyperresponsiveness in a modified version in conscious guinea-pigs. In addition to the above, the study aimed at analysing the inflammatory cell influx caused by daily (14 days) inhalation of ovalbumin (OA) in sensitized animals and determining the correlation with airway responsiveness. That kind of correlation has not been investigated so far after that length of time of repeated antigen exposure. The inflammatory nature of any changes seen in antigen-challenged guinea-pigs was investigated by pharmacological intervention with methylprednisolone.

Correspondence: Dr H. O. Heuer, Department of Pharmacology, Boehringer Ingelheim KG, D-55216 Ingelheim, Germany.

Materials and methods

Study design/protocol

Forty female Dunkin Hartley guinea-pigs (370–580 g at first sensitization) were allocated to one of four groups, consisting initially of 10 animals each. Group I consisted of completely untreated (naive) animals, whereas group II was sham-sensitized, sham-challenged and -treated as a control for groups III and IV. Groups III and IV were sensitized by 50 mg/kg s.c. plus i.p. ovalbumin (OA, 5× cryst., research grade, Serva, Heidelberg, Germany). In comparison to sham-treated OA-controls (Group III), group IV was treated with methylprednisolone-hemisuccinate (40 mg/kg i.p., 24 h and 1 h before OA-challenge and sensitization). From 3 weeks after sensitization guinea-pigs of groups III and IV were exposed for 14 days daily to inhalations of 2% OA (10 min) under cover of 0.5 mg/kg i.p. of the antihistamine mepyramine (15 min before OA). Sham-treated/-sensitized guinea-pigs were exposed to 0.9% NaCl (vehicle for OA), also under pretreatment with 0.9% NaCl (Fig. 1). Twenty-four hours after the last of the daily inhalations of OA and 24 h after another single OA inhalation (the latter under cover of 3 mg/kg i.p. mepyramine to avoid death from increased sensitivity after boosting) 1 week later airway responsiveness was tested by constructing con-

centration-response curves (CRCs) for inhaled acetylcholine (Groups II–IV). After the last CRC for acetylcholine, bronchoalveolar lavage (BAL) was performed in all groups (I–IV). In group I (naive controls) CRC for inhaled acetylcholine was only performed immediately before BAL to avoid interference of previous inhalations with BAL cell influx.

Experimental set-up for measuring airway responsiveness to inhaled acetylcholine

The conscious guinea-pigs were placed in a cylindrical double box body plethysmograph (Fig. 1). The 'head plethysmograph' had a volume of 0.57 l, the 'body plethysmograph' 1.65 l (without guinea-pig). The head in the foremost compartment was separated from the posterior compartment by a special neck seal. This consisted of two rubber pieces, adapted in size to the individual guinea-pig, held in place by a two-part rigid aluminium plate. This seal was placed in a hole between two parts of the double box and fitted around the neck of the individual guinea-pig when it was placed in the box. A pneumotachograph (Fleisch) was mounted in the wall of the body box (Fig. 1). Amplitude of respiratory flow was measured indirectly by changes in the thorax gas volume (body box) during respiration. This primary flow

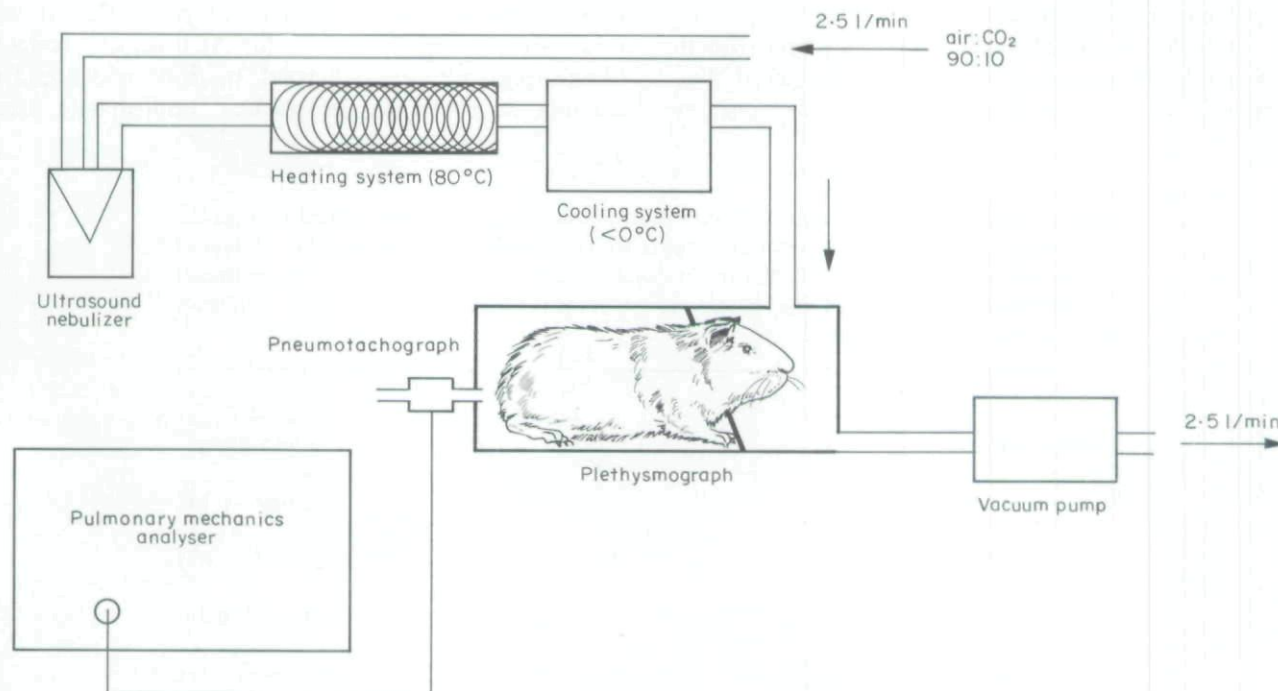


Fig. 1. Experimental setup for delivery of aerosols (bronchoconstrictors) and measurement of lung function in the conscious guinea-pig.

signal was transmitted via a differential pressure transducer (Validyne MP 45-14) and an amplifier to a recorder (Buxco, pulmonary function analyser, model 6). The latter derived other pulmonary function parameters (e.g. tidal volume and respiratory rate).

A constant bias flow of pressurized air (containing 10% CO₂) was driven at 2.5 l/min through the head box for several reasons: (i) variation of the CO₂ content in the respired air was avoided, thereby excluding variation in basal pulmonary function; (ii) aerosol of bronchoconstrictors, antigen or drugs could be added to the bias flow without interrupting the measurement of pulmonary function. Simultaneously the bias flow generated by the pressurized air transported the aerosol through the heating-cooling system (see below) to the animal; (iii) inhalation of 10% CO₂ increases respiratory frequency, maximal respiratory flow and tidal volume in the non-bronchoconstricted guinea-pig (basal pulmonary values; [6]). Increase in pulmonary flow resistance of acute bronchoconstriction is reflected in a more pronounced decrease of the above parameters compared with non-stimulated respiration. Thus, use of CO₂ for stimulation of respiration makes the measurement of pulmonary function more sensitive to detection of a bronchoconstriction or increase of airway resistance. Decrease of TV under CO₂-stimulated respiration correlates best with increased airway resistance [7]. Readings from guinea-pigs were only taken 10 min after positioning and adapting the guinea-pigs in the box to the CO₂-stimulation.

For the eventuality of aerosol generation the bias flow was conducted through an ultrasound nebulizer (M600, Medizinische Apparatebau G. Suchatzki, Rennerod/Ww, Germany) which could be activated on demand for

generation of an aerosol. In initial experiments aerosol condensed in the head box, which interfered with measurement of lung function. Therefore, the aerosol was conducted via the bias flow through a heating (80°C) and subsequent cooling (< 0°C) system through the head box. This resulted in an aerosol at room temperature which did not condense within the head box. As investigated in separate experiments not shown here (particle size analysis by laser-light diffraction, Malvern Master Sizer, Malvern Instruments, Herrsching, Germany) this procedure reduced particle size, ranging from 0.8 to 2.1 µm for 80% of particles.

Airway responsiveness to sequentially increasing concentrations of inhaled acetylcholine (range tested: 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 mg/ml ACh, depending on individual responsiveness) was performed by inhalation of ACh for 30 s. Guinea-pigs were allowed to return to baseline between exposures. Per cent bronchoconstriction was defined as % decrease of basal amplitude of flow or tidal volume. CRCs were constructed by linear regression for individual guinea-pigs. EC50 values (concentration which induced a 50% decrease of flow or TV) were derived from individual CRCs as a measure for airway responsiveness. CRCs and EC50s for groups of guinea-pigs were calculated as mean of individual values.

Performance and analysis of bronchoalveolar lavage (BAL)

Bronchoalveolar lavage (BAL) was performed at the timepoint after the last CRC for ACh (in all groups). Guinea-pigs were anaesthetized, tracheotomized and a cannula inserted into the trachea. Immediately after

Table 1. Mean of EC50s (effective concentration of inhaled acetylcholine, which reduces the TV by 50%) of concentration-response curves (CRC) for inhaled acetylcholine after 14 days of daily ovalbumin inhalations (1. CRC) and at day 21 (= 24 h after another single OA challenge) (2. CRC) for groups of guinea-pigs; parameter evaluated is tidal volume (TV); 95% confidence limits in brackets; *n* as in Fig. 3

Treatment group	1. CRC Tidal volume	2. CRC Tidal volume
Naive control	—	0.06 (0.03–0.14)
Sham control	0.03 (0.01–0.06)	0.04 (0.02–0.09)
OA-control	0.08 (0.03–0.22)	0.11 (0.03–0.41)
Methylprednisolone-h.s. 40 mg/kg i.p.	0.05 (0.02–0.11)	0.03 (0.01–0.11)

Table 2. Cell numbers (10^6 /lung) in BAL of guinea-pigs; mean $\pm$ SEM. $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$: significant difference from sham control; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$: significant difference of methylprednisolone from OA-control

Treatment group	Total cell count	Eosinophils	Neutrophils	Macrophages	Lymphocytes	Epithelial cells
Naive control	16.9 $\pm$ 1.46	4.37 $\pm$ 0.92	0.21 $\pm$ 0.07	10.5 $\pm$ 1.01	0.20 $\pm$ 0.02	2.29 $\pm$ 0.26
Sham control	16.2 $\pm$ 0.79	4.90 $\pm$ 0.58	0.46 $\pm$ 0.23	8.92 $\pm$ 0.32	0.21 $\pm$ 0.03	1.74 $\pm$ 0.32
OA-control	164 $\pm$ 20.8	98.7 $\pm$ 15.8	15.0 $\pm$ 2.91	46.4 $\pm$ 4.76	1.87 $\pm$ 0.66	2.16 $\pm$ 0.52
	+++	+++	+++	+++	++	
Methylprednisolone-h.s. 40 mg/kg i.p.	24.8 $\pm$ 3.93	7.11 $\pm$ 1.52	1.16 $\pm$ 0.40	14.5 $\pm$ 2.21	0.35 $\pm$ 0.09	1.72 $\pm$ 0.44
	***	***	***	*** ++	*	

killing the animals bronchoalveolar lavage (BAL) was performed with 3×10 ml/kg 0.9% NaCl at 37°C (15 s of gentle massage of the thorax each).

The absolute cell number was calculated from an aliquot counted in a Neubauer chamber. The differential cell counts (neutrophils, eosinophils, lymphocytes, macrophages, and epithelial cells) were analysed on a cytopspin-preparation on the basis of at least 300 cells.

Eosinophil peroxidase (EPO) as measure of eosinophil activation was analysed by a colourimetric assay modified from Strath, Warren and Sanderson [8]. Briefly, a substrate of 0.1 mM *o*-phenylenediamine dihydrochloride (OPD, Sigma Chemical Company, St Louis, MO, USA) in Tris buffer at pH 8.0 (0.05 M Tris/Tris HCl buffer) containing 0.1% Triton X 100 (Sigma) and 1 mM hydrogen peroxide was made up. Substrate solution (0.5 ml) was added to 0.5 ml of BAL supernatant fluid and kept at room temperature for 30 min in the dark. The reaction was stopped by addition of 0.25 ml of 4 M H_2SO_4 . Absorbance was read at 490 nm. EPO activity was confirmed by inhibition with 3-amino-1,2,4-triazole at 2 mM.

MPO assay was performed in a modification of the method described [9]. Briefly, 0.5 ml of supernatant from BAL were added to 0.5 ml of the reaction mixture consisting of 100 mM potassium phosphate buffer (pH 6.0), 1 mM *o*-dianisidine dihydrochloride (indicator), 0.1% Triton X, 100, 2 mM 3-amino-1,2,4-triazole (AMT, inhibitor of eosinophil peroxidase was added to one of three samples for specificity testing). After 30 min at 24°C in the dark, the optical density at 450 nm was evaluated.

Statistics

If not otherwise indicated values are given as mean $\pm$ SEM. Significant differences between groups were detected by Wilcoxon tests. Alpha adjustment was performed according to Bonferroni-Holm.

Results

Airway responsiveness

Daily (for 2 weeks) inhalation of OA by sensitized guinea-pigs did not shift the CRC for inhaled ACh to the left, measured 24 h after last OA challenge (day 14, first CRC to ACh). The failure to shift CRC for ACh to the left was still true 24 h after another OA inhalation 1 week later (day 21, second CRC) (Table 1). Methylprednisolone did not interfere with the CRCs for inhaled ACh itself (Table 1).

Inflammatory cell influx and cell activation

The BAL of sham-sensitized/-challenged guinea-pigs did

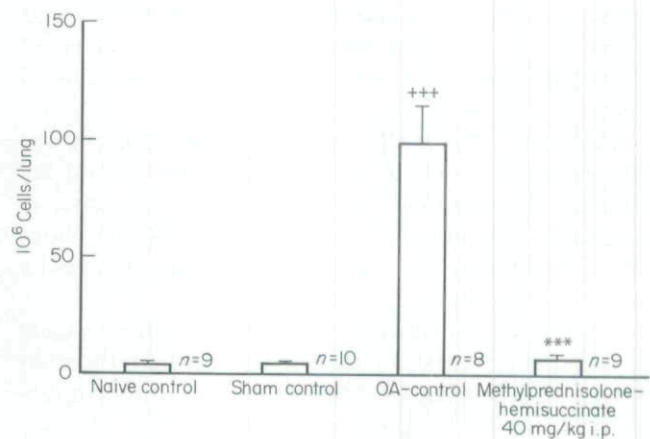


Fig. 2. Eosinophils ($\times 10^6$ /lung) in BAL 24 h after last antigen/sham-provocation (day 21 after second CRC for ACh). Methylprednisolone-hemisuccinate was dosed at 24 h and 1 h before each antigen-exposure and sensitization. Mean $\pm$ SEM; $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$: significant difference from sham-sensitized/-challenged controls; * analog significant differences from OA-controls.

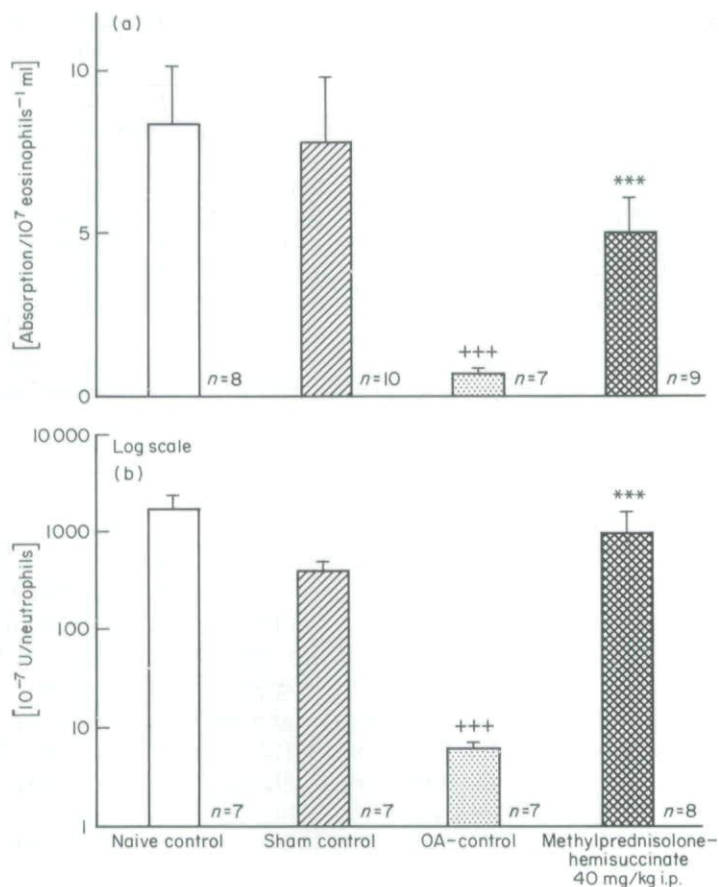


Fig. 3. Enzyme activity of EPO and MPO normalized to number of eosinophils and neutrophils, respectively in BAL from guinea-pigs 24 h after last antigen-/sham-exposure. (a) EPO/eosinophil; (b) MPO/neutrophil. Numbers as mean $\pm$ SEM. Methylprednisolone-hemisuccinate was dosed at 24 h and 1 h before each antigen-exposure and sensitization. Mean $\pm$ SEM; $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$: significant difference from sham-sensitized/-challenged controls; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$: significant differences from OA-controls.

not reveal any relevant nor statistically significant difference compared with untreated, naive guinea-pigs (Table 2, Fig. 2). There was only a trend for increased neutrophil number in sham-sensitized/-challenged animals.

BAL from repeatedly sensitized/challenged guinea pigs showed not only a significant increase of total cell count by about tenfold, but also significant increases in eosinophils by about 20-fold, neutrophils by about 30-fold, lymphocytes by about tenfold, macrophages by about fivefold (Table 2, Fig. 2).

Treatment with methylprednisolone hemisuccinate (40 mg/kg i.p.) almost completely abrogated the OA-associated cell-influx ($P < 0.001$, $P < 0.05$ for lymphocytes) (Table 2, Fig. 2).

As the number of eosinophils and neutrophils were markedly increased by antigen challenge, EPO and MPO alone do not provide an adequate marker of eosinophil and neutrophil activation, respectively. Instead the activity of EPO and MPO should be normalized to the number of eosinophils and neutrophils. Therefore in this study the ratio EPO/eosinophil and MPO activity/

neutrophil was employed. This ratio was significantly reduced in the OA-control group in comparison with the sham controls (Fig. 3). Methylprednisolone prevented this effect (Fig. 3).

Discussion

Airway hyperreactivity is one of the hallmarks of bronchial asthma. This study addressed the question whether daily inhalation of the specific antigen to sensitized guinea-pigs would not only attract and activate inflammatory cells into the lungs but concomitantly increase airways responsiveness to a bronchoconstrictor like acetylcholine.

For testing airway responsiveness a novel method for continuous and repeated measurement of lung function in the conscious guinea-pig was used. This method combines several modifications in the experimental set-up to achieve the possibility of continuously monitoring normal lung function and changes of this in case of bronchoconstriction. A bronchoconstrictor can be introduced into the system as non-condensing aerosol without

interrupting the measurement. In addition, the bias air flow allows provision of not only non-condensing aerosol, but also the stimulation of respiration by CO₂ guarantees a measurement of lung function with low variability and makes the otherwise low amplitude of respiratory flow sensitive to detection of bronchoconstriction.

The results indicate that the CO₂-induced increase of maximal respiratory flow and respiratory rate is very reproducible for each animal. The increase of respiratory flow (as well as the derived tidal volume) and respiratory rate (up to 200/min; not shown) by CO₂ is in line with the data reported for the guinea-pig [6, 10]. The complete return of respiratory flow to baseline value (increased by CO₂) after inhalation of the bronchoconstrictor ACh during establishing concentration-response curves confirms the good reproducibility of measurements.

Inhalation of ACh in increasing concentrations induced a reproducible and concentration-related decrease of the stimulated respiratory flow. It has to be considered that changes of flow resistances in whole animals may reflect changes of resistances in the upper (naso-pharyngeal) as well in the lower (intrapulmonary) airways. There are, however, several points of evidence which support the concept that measured decrease of tidal volume under CO₂-stimulated respiration indeed reflects more increased airway resistance in the lower airways. First, the nebulizer used together with the heating-cooling system guarantees the generation of ACh aerosols with more than 90% of particles smaller than 2.1 µm in diameter (not shown). In view of the stimulated respiration with deep inspirations this will make it likely that the greater amount of particles will deposit in the smaller airways. Second, acetylcholine (in contrast to histamine) practically only induces bronchoconstriction in the lower airways [11,12], thus the concentration-dependent decrease of air flow may indeed reflect bronchoconstriction in the lower airways. Finally, the fast onset and the complete reversibility of acetylcholine-associated decrease of tidal volume argues in favour of a smooth muscle contracting effect of this agonist in the lower airways. The reversibility makes also interference from secretions into the airways unlikely. Taken together it is more likely that the observed decrease of tidal volume during inhalation of acetylcholine may occur in the lower airways. However, interference from changes in the upper airways cannot be ruled out and is certainly a limitation of this study design and method for monitoring airway resistance.

Repeated and high load OA-challenge in sensitized guinea-pigs failed to induce a sustained airway hyperresponsiveness to inhaled acetylcholine in this guinea-pig model. If anything, there was a tendency to a shift to the

right in the OA-control group (group III; not significant; Fig. 3). This result is in contrast to results of Ladenius *et al.* (1990) [5] who reported a significant increase in airway responsiveness 24 h after daily OA-challenge (for 10 days) in similarly sensitized guinea-pigs. Whether the use of anaesthesia [5] and other modifications account for the difference remains to be clarified.

The lack of effect of repeated 0.9% NaCl exposure and other manipulations in sham-controls of BAL cell composition including neutrophils (compared with untreated guinea-pigs) excludes an unspecific effect by the repeated inhalation procedure on the BAL cell count. In addition, this stresses the selective induction of cell accumulation in the BAL by the specific antigen in the OA-control.

The tremendous changes seen in BAL cell composition even 1 week after cessation of daily OA challenges may reflect the high load antigen exposure over time. Furthermore, this opens a window for studying and detecting the effect of anti-chemotactic drugs like glucocorticoids.

A complete dissociation between a massive inflammatory cell influx and changes in airway hyperresponsiveness, as observed in the present study, may have several possible explanations. First, homeostatic mechanisms have to be considered which may have compensated for increased mediator production. For example, increased production of NO radicals or intrinsic release of beta-receptor stimulating adrenaline and noradrenaline may have balanced the pro-hyperreactive stimuli. Though the time-course of occurrence of these compensating mechanisms during the very early phase after antigen-challenge argues against these particular mediators, other more sustained and even unknown compensating mechanisms including neural control mechanisms cannot be ruled out. Second, exposure to antigen for 14 days may not be sufficient to induce smooth muscle hypertrophy as a feasible pathophysiologic prerequisite for a long-lasting airway hyperresponsiveness, but repeated exposures over longer time periods at fortnightly intervals may induce hyperresponsiveness in preprimed animals [13]. Also the massive inflammatory cell influx into the airway lumen together with mucus hypersecretion and mucus plugs at the time point of testing might have hindered the access of inhaled acetylcholine to the bronchial smooth muscle and compensated mechanically for a per se existing hyperreactivity [14]. In favour of the latter interpretation would be the finding that basal TV at least 24 h after 14 days of daily ovalbumin inhalations was significantly reduced in the OA-control group (TV: 3.97 ± 0.24 in sham vs 3.15 ± 0.21 ml in OA-controls, $P < 0.05$). Furthermore, decreased cholinergic reflex mechanisms after repeated antigen-inhalation may have contributed to a compensated airway responsiveness [15].

As even a massive inflammatory cell influx did not necessarily result in airway hyperresponsiveness this may question the causative role of these cells in this process [2, 16]. In fact, also under clinical conditions, it has been shown that steroids were capable of completely suppressing signs of inflammation as reflected by suppressed influx of inflammatory cells but airway hyperresponsiveness persisted [16,17]. This also supports the view of a dissociation between inflammatory cell influx and airway hyperresponsiveness. The relative higher increase of neutrophils (about 30-fold) after this repeated antigen-challenge may be consistent with a suggested role of neutrophils in the repair and recovery process from increased hyperresponsiveness [13,18]. Thus, the higher number of neutrophils might have prevented the development of airway hyperresponsiveness. On the other hand eosinophils and their release products have been assigned a major role in the development of airway hyperresponsiveness [3,4,19,20]. In this study there was no apparent increase in eosinophil activation at the time point of assessing airway responsiveness. Possibly the lack of activation can be explained by a previous activation event which could be blocked by methylprednisolone.

This is the first investigation reporting such dramatic inflammatory cell influx after daily antigen inhalation. In spite of these massive inflammatory changes this has no impact on airway responsiveness as measured with a new and sensitive method. In conclusion, the dissociation between the massive inflammatory cell influx into the BAL on the one hand and decreased activation status of these (probably previously activated, but presently exhausted) cells on the other hand suggests the hypothesis that absence of measured airway hyperresponsiveness is related to decreased activation status of the inflammatory cells. More work is needed to dissect this and other possible explanations for the dissociation observed.

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References

- 1 Ishida K, Kelly LJ, Thomson RJ, Beattie LL, Schellenberg RR. Repeated antigen challenge induces airway hyperresponsiveness with tissue eosinophilia in guinea pigs. *J Appl Physiol* 1989; 67:1133-9.
- 2 Boushey HA, Holtzmann MJ. Experimental airway inflammation and hyperactivity: searching for cells and mediators. *Am Rev Respir Dis* 1985; 131:312-3.
- 3 Gundel RH, Gerritsen ME, Wegner CD. Antigen-coated sepharose beads induce airway eosinophilia and airway hyperresponsiveness in cynomolgus monkeys. *Am Rev Respir Dis* 1989; 140:629-33.
- 4 Gundel RH, Gerritsen ME, Gleich GJ, Wegner CD. Repeated antigen inhalation results in a prolonged airway eosinophilia and airway hyperresponsiveness in primates. *J Appl Physiol* 1990; 68: 779-86.
- 5 Ladenius ARC, Campbell JW, Sivaprasad S, Suresh M, Biggs DF. Active Immunization against substance P prevents the development of airway hyperresponsiveness in guinea-pig model of asthma. *Am Rev Resp Dis* 1991; 143 (part 2):A614.
- 6 Wong KL, Alarie Y. A method for repeated evaluation of pulmonary performance in unanesthetized, unrestrained guinea pigs and its application to detect effects of sulfuric acid mist inhalation. *Toxicol Appl Pharmacol* 1982; 63: 72-90.
- 7 Schaper M, Alarie Y. The effects of aerosols of carbamylcholine, serotonin and propanolol on the ventilatory response to CO₂ in guinea pigs and comparison with the effects of histamine and sulfuric acid. *Acta Pharmacol Toxicol* 1958; 56:244-9.
- 8 Strath M, Warren DJ, Sanderson CJ. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J Immunol Methods* 1985; 83:209-15.
- 9 Bradley PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982; 78:206-9.
- 10 Matijak-Schaper M, Wong KL, Alarie Y. A method to rapidly evaluate the acute pulmonary effects of aerosols in unanesthetized guinea pigs. *Toxicol Appl Pharmacol* 1983; 69:451-60.
- 11 Holroyde MC, Norris AA. The effect of ozone on reactivity of upper and lower airways in guinea-pigs. *Br J Pharmacol* 1988; 94:938-46.
- 12 Murlas C, Lee HK, Roum JH. Indomethacin increases airway reactivity after exposure to subthreshold ozone levels. *Prostaglandins Leukotrienes Med* 1986; 21:259-68.
- 13 Gundel RH, Wegner CD, Letts LG. The onset and recovery from airway hyperresponsiveness: relationship with inflammatory cell infiltrates and release of cytotoxic granule proteins. *Clin Exp Allergy* 1992; 22:303-8.
- 14 Roum JH, Murlas C. Ozone-induced changes in muscarinic airway reactivity by different testing methods. *J Appl Physiol Respirat Environ Exercise Physiol* 1984; 57: 1783-9.
- 15 Folkerts G, Henricks PAJ, Slootweg PJ, Nijkamp FP. Endotoxin-induced inflammation and injury of the guinea-pig respiratory airways cause airway hyperactivity. *Am Rev Respir Dis* 1988; 137:1441-8.
- 16 Chapman ID, Foster A, Morley J. The relationship between inflammation and hyperactivity of the airways in asthma. *Clin Exp Allergy* 1993; 23:168-71.
- 17 Lundgren R, Soderberg M, Horstedt P, Stenling R. Mor-

- phological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *Eur Respir J* 1988; 1:883-9.
- 18 Murlas C, Roum JH. Airway hyperreactivity occurs in steroid-treated guinea pigs depleted of leukocytes by cyclophosphamide. *J Appl Physiol* 1985; 58:1630-7.
- 19 Frigas E, Gleich GJ. The eosinophil and pathophysiology of asthma. *J Clin Allergy Immunol* 1986; 77:527-37.
- 20 Gundel RH, Letts GL, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991; 87:1470-3.

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