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Characterisation of a novel airway late phase model in the sensitized guinea pig which uses silica and *Bordetella pertussis* as adjuvant for sensitization

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Abstract

The objective of the present investigation was to validate a novel model of allergic late phase reaction in the airways of conscious guinea pigs by monitoring airway function with CO_2-forced respiration. In addition airway inflammation as one possible cause for the development of airway late phase reaction was characterized by a novel technique which consists of bronchoalveolar lavage via the orotracheal route. Guinea pigs were sensitized twice at 2-week intervals with ovalbumin in silica and *Bordetella pertussis*. Two weeks after the booster sensitization all guinea pigs showed an acute decrease of tidal volume under CO_2-forced respiration 5–15 min after antigen challenge. In contrast 42 out of 68 (= 62%) screened guinea pigs exhibited airway late phase response between 4–10 h after aerosol antigen challenge. During a subsequent cross-over study methylprednisolone (twice at 16 and 1 h before ovalbumin) did not significantly interfere with the acute response. In contrast the airway late phase response as well the associated eosinophil influx into the bronchoalveolar lavage were attenuated by the steroid. In conclusion, the sensitization procedure in combination with the novel method for monitoring airway function allowed measurement of a reproducible airway late phase response in about 60% of sensitized guinea pigs. The sensitivity of exclusively the late phase response and eosinophil influx to treatment with a glucocorticoid not only correlates this model with clinical pharmacotherapy but also strengthens the inflammatory nature of this model.

Keywords: Allergic late phase; (Guinea pig); Methylprednisolone

1. Introduction

Aersosol exposure of allergic patients as well as animals to their specific antigen results in immediate airway obstruction. This immediate response is reversible. A varying proportion of asthmatic patients and animals exhibit a second longer lasting phase of airway obstruction, a so-called late phase response which occurs from 4 up to 24 h after antigen (O'Byrne et al., 1987; Larsen, 1987). The latter has been described to be less sensitive to bronchodilators (Hergardt et al., 1981; Cockcroft and Murdock, 1987; Iwama et al., 1991) and to require corticosteroids for resolution.

The mechanisms which control development of the late phase response are not well understood, nor is the reason why only a certain percentage of asthmatics and sensitized animals develop late phase response. Sensitivity of late phase response to inhibition by corticosteroids makes airway inflammation one likely contributor to the development of late phase response (Cockcroft and Murdock, 1987; Iwama et al., 1991). However, more recently also long-acting β_2-adrenoceptor agonists have been described to interfere with the late phase response (Pedersen et al., 1991; Twentyman et al., 1991).

The aim of the present study was to develop a new model for induction and monitoring airway late phase response in the conscious guinea pig. The use of silica as adjuvant for sensitization with ovalbumin was rationalized by its known capability to generate and release cytokines such as interleukin-1 (DuBois et al., 1989; Moseley et al., 1988) which in turn as proinflammatory stimuli may enhance the chance for development of late phase response. The use of a double box plethysmograph allowed not only the repeated measurement of airway function in the con-

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scious guinea pig for this kind of study design, but also allowed organization of a screening phase for identifying late phase responders. Furthermore a cross-over study design provided the possibility for testing the reproducibility of late phase response in identified late phase responders and to study the therapeutic interference by treatments such as glucocorticoids. A modification of CO_2-forced respiration as described by Wong and Alarie (1982) was used as a sensitive method for detection of airway obstruction. In addition airway inflammation as one possible cause for development of airway late phase reaction was investigated by a novel technique which consists of bronchoalveolar lavage via the orotracheal route.

2. Materials and methods

2.1. Study design / protocol

Male guinea pigs (Dunkin-Hartley Pirbright white, Interfauna, Tuttlingen, Germany; weight range 250–350 g at first sensitization) were immunized twice with a 2-week interval between sensitizations with 20 and 10 μg/kg ovalbumin, respectively, suspended in saline. *Bordetella pertussis* (0.1 ml/kg, 150 OU = opalescence units; Behringwerke, Marburg, Germany) and silica (particle size 0.007 μm; 50 mg/kg; washed in ethanol and aqua demineralisata; Sigma, St. Louis, MO, USA) were thoroughly mixed with the antigen suspension and served as adjuvant. Sensitization was performed by administration of the mixture at 2 ml per kg by the i.p. route. Another group of age-matched animals was sham-sensitized with saline.

Two weeks after booster sensitization a screening phase for the presence of a late phase response was started. After a 7 min adaptation phase in the double box plethysmograph lung function was monitored (see below) for 2 min (basal value). Under cover of the antihistamine mepyramine (10 mg/kg i.p. 15 min before antigen) sensitized guinea pigs were given inhaled ovalbumin (1%) for 2 min (for a description of the aerosol device, see below) and lung function was monitored again at 5–15 min, and at 2, 4, 5, 6, 7, 8, 9, 10 and 24 h after antigen. Sham-sensitized animals were exposed to inhalation of saline, also under pretreatment with mepyramine. All sensitized guinea pigs were exposed to inhalational antigen at 2–3-week intervals.

In a subsequent cross-over study the effect of methylprednisolone (Sigma; in 5% Solutol HS 15 (BASF, Ludwigshafen, Germany)) plus 0.5% methylcellulose in water, dosed at 40 mg/kg p.o. twice at 16 h and 1 h before antigen, on the peak of the acute response (5–15 min) and during the late phase response was investigated. Separately the effect of methylprednisolone on inflammatory cell influx 24 h after antigen was investigated during a later cross-over study.

2.2. Experimental set-up for measuring pulmonary function during immediate and late phase response in the conscious guinea pig

The conscious guinea pigs were placed in a cylindrical double box body plethysmograph. The 'head plethysmograph' had a volume of 0.57 l, the 'body plethysmograph' of 1.65 l. 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 round the neck of the individual guinea pig when it was placed in the box. A pneumotachograph (Fleisch No. OO) was mounted in the wall of the body box and head box. Respiratory flow in the body plethysmograph was measured indirectly by changes in the thorax gas volume (body box) during respiration. These primary flow signals were transmitted via a differential pressure transducer (Validyne MP 45-14 (± 2 cmH_2O) and an amplifier to a data acquisition/analysis system (Biowindows, software version 1.10, from Modular Instruments, Malvern, PA, USA). This system allowed recording and analysis of all raw data (respiratory flow) and derived parameters of pulmonary function (e.g., tidal volume, respiratory rate, minute volume, inspiratory time, expiratory time). For single data points, the default for averaging data was set at 5 breaths. A printout of all basic data in the form of a summary table and data files for further evaluation was produced.

A constant bias flow of pressurized air (containing 10% CO_2) was driven at 2.5 l/min through the head box for several reasons: (1) variation of the CO_2 content in the respired air was avoided, thereby excluding variation in basal pulmonary function; (2) inhalation of 10% CO_2 increased respiratory frequency, maximal respiratory flow and tidal volume in the non-bronchoconstricted guinea pig (basal pulmonary values; Wong and Alarie, 1982). Increase in pulmonary flow resistance or acute bronchoconstriction was reflected in a more pronounced decrease in the above parameters compared to non-stimulated respiration. Thus use of CO_2 for stimulation of respiration makes the measurement of pulmonary function more sensitive to detection of a bronchoconstriction or increase of airway resistance. Decrease of tidal volume under CO_2-stimulated respiration correlates best with increased airway resistance (Matijak-Schaper et al., 1983; Schaper and Alarie, 1985). Readings from guinea pigs were only taken 10 min after positioning and adapting the guinea pigs in the box under the CO_2 stimulation.

For the eventuality of aerosol generation the bias flow was conducted through an ultrasound nebulizer (M 600, 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 generated a particle size ranging from 0.8 to 2.1 μm for 80% of the particles.

2.3. Performance and analysis of bronchoalveolar lavage

In separate experiments 7 guinea pigs (initially identified as having no late phase response) as sensitized and challenged here and after two more antigen challenges at 2- to 3-week intervals were studied for the effect of methylprednisolone (regimen as above) on bronchoalveolar cell influx 24 h after antigen inhalation. Bronchoalveolar lavage (2×2 ml saline) was performed via the orotracheal route under anaesthesia with ketamine (Ketavet, Parke Davis, Freiburg, Germany; 100 mg/kg i.p.)/xylazine (Rompun, Bayer, Leverkusen, Germany; 4 mg/kg i.p.). Eight additional guinea pigs were lavaged without previous antigen inhalation to compare basal cell number of sensitized animals with sham sensitized animals.

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 cytospin preparation on the basis of at least 300 cells.

Eosinophil peroxidase (EPO) as measure of eosinophil activation was analysed by a colorimetric assay modified from Strath et al. (1985). Briefly, a substrate of 0.1 mM *o*-phenylenediamine dihydrochloride (OPD, Sigma) 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 bronchoalveolar lavage 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 (AMT) at 2 mM.

Myeloperoxidase (MPO) assay was performed in a modification of the method described by Bradley et al. (1982). Briefly, 0.5 ml of supernatant from bronchoalveolar lavage was 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.

2.4. Analysis of late phase response and statistics

Late phase response during cross-over studies was present by definition if during the follow-up of lung function between 4–10 h (7 time points) tidal volume decreased (1) at least once below 70% of basal and (2) at least 4 times (majority of time points) below 75% of basal value.

Furthermore, animals under treatment with methylprednisolone during the cross-over study were only evaluated if (1) basal value of tidal volume of individual guinea pigs was higher than 3.5 ml; (2) the smaller of both basal values did not deviate by more than 25% from the higher one; (3) during the control/vehicle phase of cross-over animals had a late phase response according to the above definition.

For statistical evaluation of the late phase response the median of the values between 4–10 h for each individual animal was used to reduce for possible variability between time points of measurements. For graphic presentation tidal volume was continued to be presented as mean ± S.E.M. The other parameters of pulmonary function during the screening phase were evaluated and presented as mean ± S.E.M. at the time points 2 and 24 h, but as median for the time period between 4–10 h.

Sham treated controls were for statistical reasons (comparison with cross-over treatment groups) allocated at random to measurement A or B. For evaluation of late phase response an analysis of variance was performed. If during the interval of analysis for late phase response the ovalbumin control phase was significantly different from the measurement A of sham treated animals, the effect of treatment (methylprednisolone) was compared by paired Wilcoxon test to the ovalbumin control phase.

3. Results

3.1. Measurement of lung function, immediate and late phase response

Under cover of mepyramine all sensitized guinea pigs still developed a pronounced immediate response between 5–15 min. Measurement of tidal volume in combination with 10% CO_2-forced respiration proved to be a sensitive parameter for detection of airway resistance in spontaneously breathing and conscious guinea pigs.

Out of 68 screened guinea pigs 42 ($= 62\%$) developed a late phase response according to the above definition between 4–10 h after antigen inhalation (Fig. 1). Tidal volume continued to be significantly decreased from sham controls 24 h after antigen inhalation. In contrast, the remainder of the sensitized guinea pigs did not fulfill the criteria for presence of late phase response (Fig. 1). In contrast, sham sensitized/challenged animals did not devi-

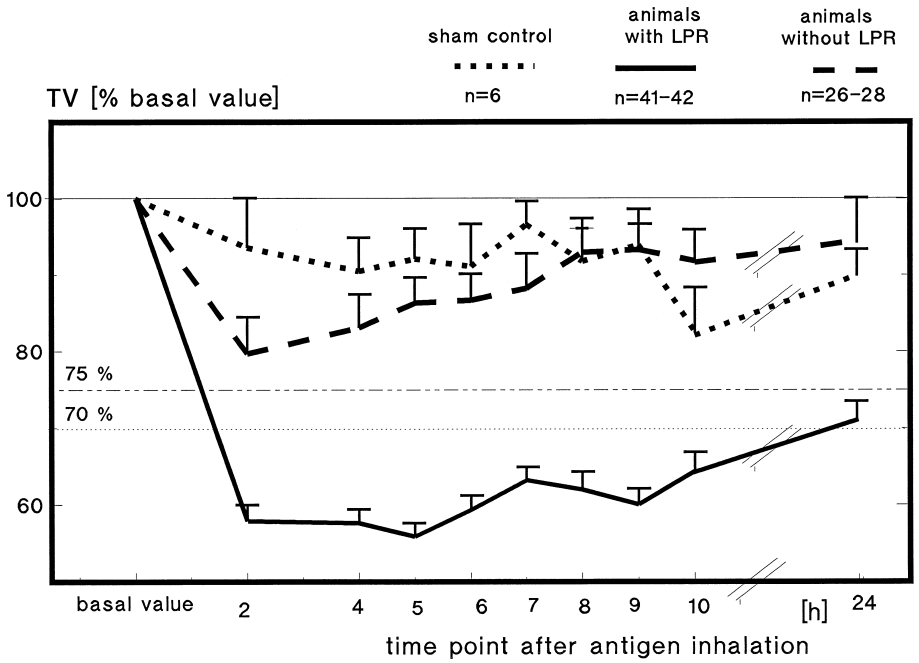

Fig. 1. Time course of tidal volume during screening for late phase response, tidal volume expressed in percentage of basel in mean + S.E.M. Criteria for presence of late phase response: see Section Section 2 ($n = 42$; $n = 41$ for 4 and 24 h); guinea pigs without late phase response did not fulfill those criteria (0–2 h: $n = 28$; 4 h: $n = 26$).

ate in the peak of the tidal volume during immediate response or during the late phase time interval significantly from basal. This implicated that they did not fulfill the definition for presence of late phase response.

In parallel with decreased tidal volume also minute volume and maximal respiratory flows were significantly decreased at 2 h and during the late phase response interval between 4–10 h. During the late phase response there was also a significant increase of respiratory rate (increase of median by about 20%).

The late phase response in guinea pigs previously identified as late phase responders was reproducible in the control phase of the cross-over study. Only one guinea pig did not reproduce late phase response during reinvestigation during this cross-over immediately after the screening phase. Other animals had to be excluded from evaluation because of differing basal values or because they died during the study.

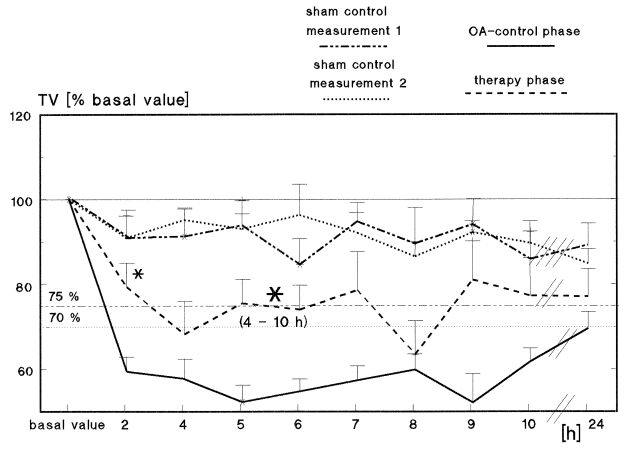

Fig. 2. Inhibition of late phase response (decrease of tidal volume) by oral methylprednisolone at 40 mg/kg, administered p.o. at 16 and 1 h before ovalbumin inhalation. Time course of tidal volume, expressed in percentage of basal; mean + S.E.M. Guinea pigs which during the ovalbumin control phase did not fulfill criteria for late phase response were excluded from evaluation. For statistical evaluation the time points at 2 and 24 h were considered separately. During the time interval for late phase response between 4–10 h the median was evaluated. $P < 0.05$: significant difference of methylprednisolone treatment from ovalbumin control phase.

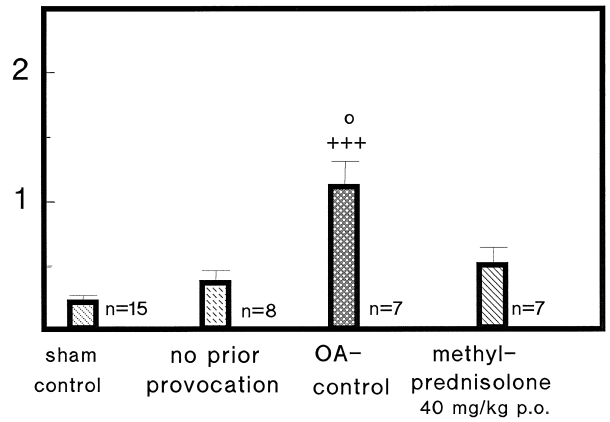

Fig. 3. Eosinophils ($\times 10^6$/lung) 24 h after ovalbumin/sham inhalation in guinea pigs. Mean + S.E.M. Bronchoalveolar lavage was performed by 2×2ml saline via the orotracheal route. $^{+++}$ $P < 0.001$: significant difference of ovalbumin control to sham; $^{\circ}$ $P < 0.05$: significant difference of ovalbumin control to sensitized guinea pigs without ovalbumin inhalation 24 h before bronchoalveolar lavage.

Methylprednisolone did not interfere with the maximal decrease of tidal volume during the immediate response between 5–15 min. In contrast methylprednisolone inhibited the antigen induced decrease of tidal volume significantly at 2 h (by 62%) and the late phase response between 4–10 h by 52% (Fig. 2).

3.2. Bronchoalveolar lavage

Recovery of bronchoalveolar lavage fluid by this novel technique varied between 66.6 ± 1.2% (sensitized guinea pigs without challenge, $n = 8$), 67.0 ± 1.5% (sham, $n = 15$), 68.9 ± 2.1% (ovalbumin control, $n = 7$) and 71.4 ± 0.7% (methylprednisolone, $n = 7$). Total cell number (total), eosinophils, neutrophils (PMNs) and macrophages were significantly increased 24 h after ovalbumin inhalation ($\times 10^6$/lung: total 4.4 ± 0.5, eosinophils 1.1 ± 0.2, PMNs 1.4 ± 0.16, macrophages 1.6 ± 0.18, respectively), when compared to both sham control (total 1.3 ± 0.1, eosinophils 0.2 ± 0.03, PMNs 0.1 ± 0.04, macrophages 0.7 ± 0.07) and sensitized guinea pigs without challenge (total 2.0 ± 0.4, eosinophils 0.35 ± 0.1, PMNs 0.33 ± 0.28, macrophages 0.88 ± 0.09). The absolute neutrophil number was the parameter for which increase was most pronounced (about 14-fold). Methylprednisolone significantly inhibited the antigen-associated influx of eosinophils 24 h later by 54%, but did not reduce all other cell types (Fig. 3).

When analyzing enzyme activity in the supernatants from bronchoalveolar lavage fluids, the absolute MPO concentration was increased. In addition the EPO concentration, normalized to the number of eosinophils, significantly decreased (ovalbumin controls vs. sham, Fig. 4).

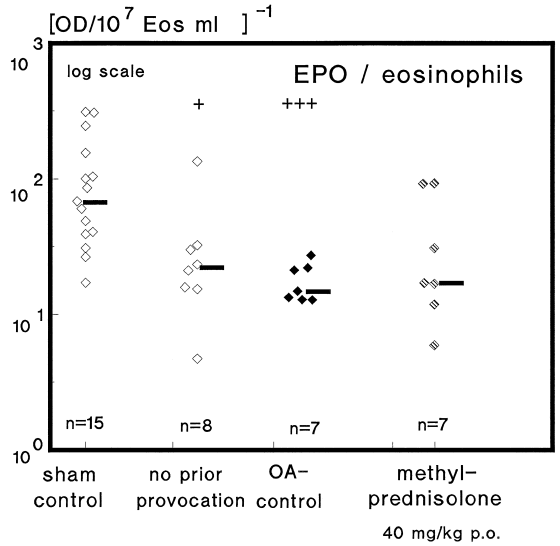

Fig. 4. EPO/eosinophil as a marker for cell activation 24 h after ovalbumin/sham inhalation in guinea pigs. Absolute values and median. $^{+}$ $P < 0.05$, $^{+++}$ $P < 0.001$: significant difference of ovalbumin control to sham.

Although with respect to absolute cell numbers sham controls did not significantly differ from sensitized guinea pigs without challenge, EPO/eosinophil was also significantly decreased in sensitized guinea pigs without preceding challenge (Fig. 4).

4. Discussion

The present work describes a novel model for investigation of airway allergic late phase response in the conscious guinea pig. The non-invasive approach by this method for monitoring pulmonary function and the avoidance of anaesthesia offer not only the convenient possibility of repeated measurements in the same animal but also the possibility of cross-over studies in previously identified late phase responders. Furthermore there is also no possibility for a putative interaction of anaesthesia with the late phase response (Brattsand et al., 1985; Iijima et al., 1987). Although the mechanisms governing the late phase response are still unclear it is evident that this dual phase is at least timely associated with an increased inflammatory cell influx, recovered in the bronchoalveolar lavage fluid and in airway tissue (Iijima et al., 1987; Gundel et al., 1992).

The use of silica and *Bordetella pertussis* as adjuvant for sensitization against ovalbumin was rationalized from the known effect of silica (DuBois et al., 1989; Moseley et al., 1988) and endotoxin (as provided by *B. pertussis*; Männel et al., 1987) to modulate the release of cytokines, including tumour necrosis factor (TNF) and interleukins, and antibody formation. TNF (Broide et al., 1992) and different interleukins (Robinson et al., 1992) have been described to be associated with chronic inflammation and priming in asthma. The continuing inflammatory effect of these cytokines may be speculated to be one contributor to the pulmonary late phase response after antigen (Kanehiro et al., 1992).

The high incidence (about 60% of investigated guinea pigs), the pronounced extent and reproducibility of the late phase response support the view that the use of silica in combination with *B. pertussis* as adjuvant are critical for the induction of late phase response in this model. This is supported by our failure to detect a late phase response in guinea pigs when sensitized against ovalbumin alone or in combination with aluminium hydroxide as adjuvant under otherwise similar conditions of monitoring late phase response (results not shown). Furthermore, because of the obvious involvement of cytokines induced by silica, the late phase response in this model may be of inflammatory nature. All investigated guinea pigs developed an immediate response, thus confirming the successful sensitization procedure. However, the reason why only a certain percentage of guinea pigs developed late phase response is still unclear. Depending on the sensitization regimen there

are only few reports on the development of a pulmonary late phase response in the guinea pig (Iijima et al., 1987; Inoue et al., 1992) showing an incidence of late phase response varying between 40–80%. The higher incidence of late phase response in guinea pigs which in their sensitization regimen had silica as adjuvant (80%; Inoue et al., 1992) is in line with our finding. The incidence as found here is also in agreement with the likelihood for development of late phase response in other species (sheep: 65%; Abraham et al., 1988) and man (Hargreave, 1989; Durham et al., 1984; Metzger et al., 1986; Rossi et al., 1991; Venge and Dahl, 1989). In human asthma the chance for development of late phase response varies between 36% (Rossi et al., 1991) and 77% (Venge and Dahl, 1989).

CO_2-stimulated respiration made measurement of the amplitude of respiratory flow and tidal volume sensitive to detection of increased airway resistance (Wong and Alarie, 1982; Matijak-Schaper et al., 1983; Schaper and Alarie, 1985). When compared to other parameters of pulmonary function during the screening phase, decreased tidal volume turned out to be the most sensitive and powerful parameter for evaluation of an airway late phase response. This is also because other parameters of pulmonary function evaluated (like minute volume, inspiratory and expiratory time) are very much dependent on respiratory rate. The latter was during late phase response significantly increased which in turn may reflect a partial compensation of the decreased tidal volume during late phase response. The trend for normalization of decreased minute volume during late phase response supports this view although minute volume was still significantly decreased and thereby late phase response was still present and functionally relevant. In contrast, the quality of changes in respiratory pattern at 2 h was different from those during the late phase response: respiratory rate was unchanged, thus the extent of decreases of minute volume, inspiratory and expiratory time, peak of flow changes were parallel and consistent to decreases of tidal volume.

The immediate response after antigen in the guinea pig and other species is predominantly mediated by histamine. This necessitated the use of a high dose of the antihistamine mepyramine to prevent immediate death after antigen challenge. The remaining pronounced decrease of tidal volume 5–15 min after antigen thus reflects the histamine-independent part of the immediate response. The failure of the glucocorticoid methylprednisolone to interfere with the immediate response is in accordance with results in man and animals (Booij-Noord et al., 1971; Cockcroft and Murdock, 1987; Chand et al., 1990), although after a longer time period of treatment with glucocorticoid some inhibition in about 50% of allergic patients could be demonstrated. Also in some animal models including guinea pigs (Andersson et al., 1988; Iwama et al., 1991) and sheep (Delehunt et al., 1984) glucocorticoids inhibited to some extent the immediate response if the time interval between pretreatment and antigen challenge was long enough. Although in this model the treatment regimen used here included twice dosing and a longer time interval before antigen challenge, the maximal decrease of tidal volume between 5–15 min after antigen was not influenced. On the other hand, the evaluation of the maximal decrease of tidal volume does not exclude an effect of glucocorticoids on the duration or course of the immediate response which was not considered here.

Exclusive inhibition of late phase response in this novel model is in accordance with other findings in animals and man: in allergic patients glucocorticoids inhibited the late phase response but not the early phase (Booij-Noord et al., 1971; Cockcroft and Murdock, 1987). In the sheep not only inhibition of late phase response but also of late phase response associated eosinophil influx into the airways was inhibited even via therapeutic intervention by methylprednisolone 3 h after antigen (Abraham et al., 1988). Also in other species late phase response as well as eosinophil influx were inhibited by glucocorticoids (Chand et al., 1990; Iwama et al., 1991; Gulbenkian et al., 1990; Andersson et al., 1988).

In the dose regimen administered it has to be emphasized that methylprednisolone in this model did neither inhibit completely the late phase response nor the antigen associated eosinophil influx. Different explanations may apply to this incomplete inhibition. Guinea pigs are particularly resistant or insensitive to treatment with glucocorticoids (Hodgson and Funder, 1978; Giannopoulos and Keichline, 1981). Furthermore it is clinical experience that asthmatics necessitate a longer lasting course of treatment with glucocorticoids before maximal improvement of symptoms occurs (Kraan et al., 1988; Barnes, 1990). Therefore it may well be that a longer lasting pretreatment period with a glucocorticoid would attenuate the late phase response in this model to a higher extent. On the other hand it cannot be excluded that the incomplete inhibition by glucocorticoids may reflect other contributing factors to the late phase response, e.g., direct bronchoconstrictive mechanisms which are insensitive to antiinflammatory treatment.

Though incomplete the relevant reduction of late phase response and antigen associated eosinophil influx by the glucocorticoid suggest the inflammatory nature of the late phase response. In contrast, total cell numbers as well other cell types like neutrophils were not affected by the glucocorticoid in guinea pigs as sensitized and challenged here. The results support some role of eosinophils in the development of late phase response. Also in man inflammatory cell influx has been associated with the occurrence of late phase response, either exclusively eosinophils (DeMonchy et al., 1986; Ädelroth et al., 1990; Rossi et al., 1991) or eosinophils and neutrophils (Metzger et al., 1986; Fabbri et al., 1987). Even some correlation between the extent of late phase response and eosinophil influx could be demonstrated (DeMonchy et al., 1986). In some animal models of late phase response eosinophil number during

late phase response was increased compared to single responder (Abraham et al., 1988; Iijima et al., 1987; Gulbenkian et al., 1990). In other animal models (monkey, Gundel et al., 1992; dog, Sasaki et al., 1987; rabbit, Murphy et al., 1986) extent of neutrophil accumulation correlated with the late phase response and suggested a causative involvement of neutrophils in the late phase response. Although neutrophils were elevated most pronounced (more than 10-fold) in similar sensitized and challenged guinea pigs used here the lacking inhibition of its influx by methylprednisolone in contrast to inhibition of late phase response does not point to a major involvement of this cell type in the late phase response in this model.

In parallel to the increased number of neutrophils the absolute MPO content in the supernatants of bronchoalveolar lavage fluids was increased. The decreased EPO content, normalized to the number of eosinophils (EPO/eosinophil), both in ovalbumin controls as well as in sensitized, but previously not challenged guinea pigs, as a marker for eosinophil activation reflects chronic ongoing activation of eosinophils as a basis for chronic inflammation in this model. Therefore the chronically maintained inflammation as indicated by decreased EPO/eosinophil also in previously (24 h before bronchoalveolar lavage) not challenged guinea pigs may provide the basis for development of late phase response in this novel model. However, a modulatory role of other inflammatory mediators and markers of inflammation, like tachykinins and edema, as well as other additive bronchoconstrictive stimuli, cannot be exlcuded in the development of late phase response.

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