

Relationships among allergen-induced early and late phase airway obstructions, bronchial hyperreactivity, and inflammation in conscious, unrestrained guinea pigs

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The relationships among allergen-induced early asthmatic reactions (EARs) and late asthmatic reactions (LARs), early (between EAR and LAR) and late (after LAR) changes in bronchial reactivity to histamine and infiltration of inflammatory cells into the airways were investigated with a new model of chronically instrumented, unrestrained, and ovalbumin-sensitized guinea pigs. Two different provocation strategies were examined. With the use of stepwise increasing allergen concentrations, all 21 animals responded with an EAR, which in 15 animals (71%) was followed by an LAR. By inhalation of a single allergen concentration for up to 15 minutes, 11 of 14 animals showed an EAR, which in 10 animals (71%) was followed by an LAR. One animal did not respond, whereas the remaining two showed only an LAR. At 6 hours (after the EAR) and 24 hours (after the LAR) after allergen provocation, a significant bronchial hyperreactivity (BHR) toward histamine aerosol was observed in the dual responding animals (both protocols), but no significant changes were observed in animals with a single EAR or a single LAR. Significant correlations were found between the initial increase in airway obstruction after allergen provocation and the severity of the EAR and LAR, as well as the early and late BHR; in addition, a significant correlation was found between the early and late BHR. In contrast, the severity of the LAR did not correlate with the BHR at 6 hours and 24 hours. At 6 hours, there was a marked tendency to an increase in the number of eosinophils and a significant increase in the number of neutrophils in the bronchoalveolar lavage. At 24 hours after provocation, the number of eosinophils and neutrophils was significantly enhanced. These data suggest that early activation of mast cells and/or inflammatory leukocytes may determine the development of the LAR, as well as the early and late BHR, although there appears to be no causal relationship between the BHR at both time points and the severity of the LAR. The relationships among allergen-induced EAR and LAR, early and late BHR, and airway inflammation observed in this new guinea pig model are strikingly similar to those observed in patients with asthma. (J ALLERGY CLIN IMMUNOL 1994;93:1021-30.)

Key words: Early and late phase airway obstructions, bronchial hyperreactivity, airway inflammation, unrestrained guinea pigs, allergic asthma

A nonspecific increase in airway responsiveness to inhaled bronchoconstrictor stimuli is a common characteristic of asthma. Allergen inhalation resulting in an early asthmatic reaction (EAR) and a late asthmatic reaction (LAR) has generally

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Abbreviations used

AUC:	Area under the percentage change in pleural pressure time curve
BAL:	Bronchoalveolar lavage
BHR:	Bronchial hyperreactivity
EAR:	Early asthmatic reaction
LAR:	Late asthmatic reaction
PC ₁₀₀ :	Provocative concentration causing a 100% increase in pleural pressure
P _{pl} :	Pleural pressure

been associated with the development of bronchial hyperreactivity (BHR).¹⁻³

The EAR is considered to result from an IgE-dependent release of preformed and newly gen-

erated mediators from resistant mast cells in the airway mucosa. On the other hand, the LAR is accompanied by recruitment of inflammatory cells, such as eosinophils and neutrophils, to the lung.^{4, 5} Granule-derived and newly formed mediators from these cells could contribute to the LAR and to the enhanced bronchial hyperresponsiveness that is usually observed after the LAR.^{1, 2} Recent investigations have indicated that enhanced BHR after allergen challenge may already be present before the LAR is clinically manifest⁶ and that this early change in BHR is correlated with the development of the LAR.^{3, 7}

Although clinically well characterized, the exact pathologic mechanisms underlying the allergen-induced LAR and early (between EAR and LAR) and late (after LAR) changes in BHR are still poorly understood. Because these mechanisms cannot be exhaustively investigated in human subjects because of experimental limitations, there has been considerable interest in the development of animal models of human asthma.⁸ Of these, the sensitized guinea pig has received considerable attention as a small laboratory animal that may develop allergen-induced early and late asthmatic responses,⁹⁻¹¹ airway inflammation¹⁰⁻¹⁷ and BHR.¹⁵⁻¹⁹ However, the relationships among these processes, especially with regard to the occurrence of allergen-induced EARs and LARs and the development of BHR has not been investigated thus far. To study these relationships in individual animals, it is necessary to measure airway functions repeatedly and continuously during prolonged periods. Most of the currently available methods for the assessment of airway function parameters in guinea pigs are not suitable for this purpose, because they are based on whole body plethysmography, in which the physical restraint impairs continuous measurements over longer periods or because they are based on invasive methods that use anesthesia, in which the chemical restraint impairs repeated measurements over longer periods. Moreover, fixation stress and anesthesia may greatly affect airway functioning.^{20, 21} Therefore we recently developed a new method to assess airway functions in chronically instrumented, conscious, and unrestrained guinea pigs.²² This method is based on on-line measurement of pleural pressure (P_{pl}) through a fluid-filled balloon, placed inside the thoracic cavity, which provides an adequate description of histamine- and allergen-induced airway obstruction.²² Using this method, we investigated the development of allergen-induced EARs

and LARs in relation to early and late changes in BHR in ovalbumin-sensitized animals. In addition, allergen-induced airway inflammation was investigated by determining the cellular contents of the bronchoalveolar lavage (BAL) fluid.

METHODS

Animals

Male specific pathogen-free guinea pigs (Charles River SAVO, Kiszlegg, Germany), weighing 500 to 700 gm, were used in this study. All animals were previously sensitized to ovalbumin at 3 weeks of age, when they weighed approximately 250 gm. A shift to the heat-labile IgE class antibodies was obtained by adding 100 mg/ml aluminum hydroxide to an allergen solution containing 100 μ g ovalbumin per milliliter saline solution.²³ The allergen solution was gently rotated for 60 minutes to obtain an aluminum hydroxide gel, and 0.5 ml was injected intraperitoneally, while another 0.5 ml was divided over seven subcutaneous injection sites in the proximity of lymph nodes in the paws, lumbar regions, and neck.²⁴ Animals were operated on in week 3 after sensitization and used experimentally in weeks 4 to 8. The animals were housed in individual cages in climate-controlled animal quarters and given water and food ad libitum. All protocols described 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.²² In brief, a small latex balloon, connected to saline solution-filled tubing, was surgically implanted in the thoracic cavity. The cannula was driven subcutaneously and permanently attached to the neck of the animal. After connection through a fluid-filled cannula to a pressure transducer (Gould P23ID, Gould Medical BV, Bithoven, The Netherlands), P_{pl} was measured in centimeters of H_2O with an on-line computer system, sampling for 10 seconds per minute. Breath-by-breath variation in P_{pl} was normally less than 15%. Incidental large variations caused by sudden movements or deep sighs were excluded from the calculations. As previously described,²² no postoperative inflammation was observed for at least 5 weeks after operation, and baseline P_{pl} values remained stable during repeated measurements on different days. Using a combination of flow measurement with a pneumotachograph, implanted in 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.²² Airway function can be registered repeatedly and continuously for periods of at least 24 hours, while the animals are unaware of the measurements taken.

Provocation techniques

For histamine and allergen aerosol provocations, an animal cage was developed, in which the guinea pigs could move freely.²² 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 DeVilbiss nebulizer (type 646; DeVilbiss, Somerset, Pa.), driven by an airflow of 8 L/min, provided the aerosol required, with an output of 0.43 ml/min.

On the first day of the experimental protocol, the animals were placed inside the provocation cage unconnected to the transducers. Three consecutive challenges with saline solution were performed; these lasted 3 minutes each and were separated by 10-minute intervals. The next day, this protocol was repeated with the animals connected to the measuring system. From then on, animals were challenged with saline solution, histamine, and ovalbumin aerosols. All provocations were preceded by an adaptation period of at least 30 minutes, followed by two consecutive control challenges with saline solution, as described above.

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

Allergen provocations were performed with two different protocols: (1) by inhalation of stepwise increasing concentrations of 0.1%, 0.3%, and 0.5% ovalbumin in saline solution for 3 minutes, separated by 10-minute intervals and (2) by inhalation of a single concentration of 0.2% ovalbumin in saline solution for up to 15 minutes. Allergen inhalations were discontinued when an increase in P_{pl} of more than 100% was observed. No antihistamines were needed to prevent anaphylactic shock.

Provocation protocol

To establish baseline histamine PC_{100} values, two histamine provocation sessions were performed, separated by 3 days. Variations between the first and second PC_{100} values were small ($3.9\% \pm 2.9\%$, $n = 39$). One day after the second histamine provocation, allergen or saline control provocations were performed. At 6 hours (between the EAR and LAR) and 24 hours (after the LAR) after these provocations, histamine PC_{100} values were reestablished 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} before and after saline or antigen provocation. On 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 hours and 24 hours, the animals were removed from the

provocation cage and placed in a larger cage of 2500 cm², where they could move around freely and eat and drink ad libitum. During this transfer, the animals remained connected to the measurement system.

BAL procedure

BAL was performed either 6 or 24 hours after allergen or saline provocation. Animals were anesthetized with 20 mg/ml methohexital, 35 mg/kg ketamine hydrochloride, and 6 mg/kg Rompun (Bayer, Leverkusen, Germany) administered intraperitoneally, which ensured a fast, deep anesthesia. The trachea was exposed, and the lungs were gently lavaged by means of a tracheal cannula with 5 ml of sterile saline solution at 37° C, followed by 3 subsequent aliquots of 8 ml of saline solution. The recovered lavage samples were cooled on ice and centrifuged at 200g for 10 minutes at 4° C. The pellets were combined and resuspended to a final volume of 1.0 ml in RPMI-1640 medium (Gibco Life Technologies, Paisley, Scotland). Total cell numbers were counted in a Bürker-Türk chamber (Tamson, Zoetermeer, The Netherlands). For cytologic examination, cytopsin preparations were made in a Shandon cytopsin-2 cytocentrifuge (Shandon Southern Instruments, Swickley, Pa.) with 100 μ l aliquots of the cell suspension, spinning at 300g for 5 minutes. The preparations were stained with May-Grünwald and Giemsa stain. 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.). Brietal-sodium (methohexital) was purchased from Eli Lilly (Amsterdam, The Netherlands), ketamine hydrochloride from Parke-Davis (Barcelona, Spain), Rompun (2-(2,6-xylylidino)-5,6-dihydro-4H-1,3-thiazine-hydrochloride, methylparaben) from Bayer (Leverkusen, Germany), and RPMI-1640 medium from Gibco Life Technologies (Paisley, Scotland).

Data analysis

The magnitude of the EAR after allergen provocation was expressed as the initial maximal increase in P_{pl} and the area under the percentage change in P_{pl} time curve (AUC) between 0 and 6 hours after provocation, calculated by trapezoid integration over 5-minute periods. Because of marked oscillations in P_{pl} during the LAR, the magnitude of the LAR was expressed only as the AUC between 8 and 24 hours after provocation. With the AUC values from saline control provocations (Table I), threshold values (mean + $2 \times$ SD) of 1185 for a positive EAR and 2790 for a positive LAR were defined. With these criteria, animals were characterized as nonresponders, single early responders, single late responders, and dual responders. Development of BHR after provocation was evaluated by a Student's *t* test for paired observations. Student's *t* test for unpaired data was used to compare data from different groups of

TABLE I. Airway responses of guinea pigs after saline or allergen provocation

Provocation procedure	n	AUC EAR (% ΔP_{pl})	max P_{pl} EAR (% ΔP_{pl})	AUC LAR (% ΔP_{pl})
Saline	5	331 $\pm$ 191	29.0 $\pm$ 13.3	532 $\pm$ 505
Allergen				
Increasing dosages				
Single EAR	6	2604 $\pm$ 557*	140.7 $\pm$ 27.2*	693 $\pm$ 436
EAR + LAR	15	3615 $\pm$ 581*	211.0 $\pm$ 37.7†	7802 $\pm$ 1540†
Continuous dosage				
Single EAR	1	5696	203.0	495
EAR + LAR	10	3397 $\pm$ 645*	198.0 $\pm$ 24.9‡	8354 $\pm$ 1339*
Single LAR	2	-984 ; 676T	30.4 ; 69.8	5655 ; 32443

Data represent mean values $\pm$ SEM. Statistical analysis was done with unpaired Student's *t* test compared with saline control. % ΔP_{pb} , Percent change from baseline P_{pl} , mean values $\pm$ SEM, being 4.01 ± 0.25 cm H₂O (*n* = 39); max P_{pl} EAR, maximal increase in P_{pl} observed during EAR; EAR, early asthmatic response during 0 to 6 hours after provocation; LAR, late asthmatic response during 8 to 24 hours after provocation.

**p* < 0.01.

†*p* < 0.05.

‡*p* < 0.001.

animals. Correlations between different parameters of airway function and BAL were determined by least squares linear regression analysis. Differences were considered to be significant when the *p* value was less than 0.05. All data are presented as means $\pm$ SEM.

RESULTS

Allergen-induced asthmatic responses

Examples of allergen-induced asthmatic responses, as well as a saline control challenge, are presented in Fig. 1. In both protocols the early responses start with a sharp increase in P_{pl} , which is maximal within 20 minutes and lasts for approximately 2 to 5 hours. The late responses start at approximately 8 hours after allergen provocation and are reversible within 24 hours after the challenge. Of the 21 animals in the first increasing dosage protocol, 6 (29%) developed a single EAR, whereas 15 (71%) responded with a dual reaction. Of the single early responders, one animal responded at 0.1% ovalbumin, three at 0.3%, and two at 0.5% (mean concentration, $0.33\% \pm 0.06\%$). Of the dual responders, seven animals responded at 0.1% ovalbumin, seven at 0.3%, and one at 0.5% (mean concentration, $0.22\% \pm 0.03\%$; not significant).

By definition, all animals responded with an EAR in this protocol. To investigate the possible occurrence of isolated late-phase reactions, a second allergen inhalation protocol was used in which a low dose of 0.2% ovalbumin was administered for up to 15 minutes. Indeed, two of the 14 animals in this group did develop a single LAR, one animal developed a single EAR, and 10

animals (71%) had a dual response. All except four animals in the dual responder group required the full inhalation time (15 minutes) to develop airway obstruction. One animal did not respond to allergen provocation and was excluded from the study.

The airway responses to allergen provocation with both protocols are presented in Table I. No differences were observed between both allergen inhalation procedures with respect to the early- and late-phase responses in single early and dual responders, respectively. In addition, no significant differences were found in AUC and maximal increase in P_{pl} of the EAR from single and dual responders in both groups, although there was a tendency toward lower values in the single responders. When all data were taken together, there appeared to be a significant correlation between the maximal change in P_{pl} (but not the AUC) of the EAR and the AUC of the LAR (*r* = 0.41; *p* = 0.009).

Allergen-induced BHR

Changes in airway reactivity to histamine at 6 hours and at 24 hours after saline or allergen provocation, expressed as the ratio of histamine PC₁₀₀ before challenge to histamine PC₁₀₀ after challenge, are presented in Table II. Neither at 6 hours nor at 24 hours after saline provocation was a significant difference from unity observed. When the increasing dosage provocation protocol was used, no significant increase in the airway reactivity to histamine was observed in the ani-

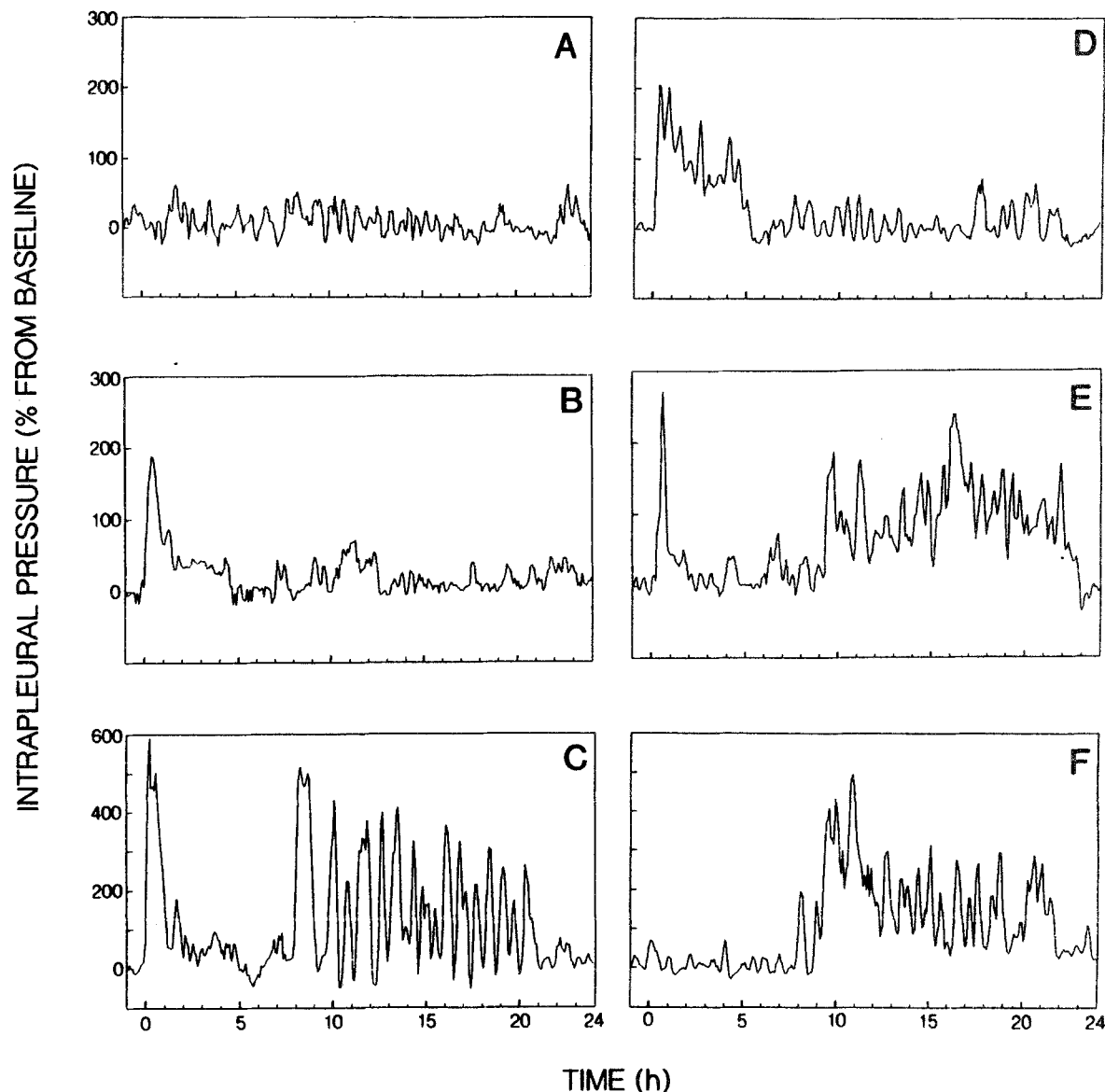


FIG. 1. Representative examples of airway responses after saline (**A**) or allergen provocation (**B-F**) of ovalbumin-sensitized guinea pigs. **B** and **C** represent a single early responder and a dual responder, respectively, when the increasing dosages protocol was used. **D**, **E**, and **F** represent single early, dual, and late responders, respectively, when the continuous dosage protocol was used. Provocations were performed at 0 hour. For improved visualization the responses are presented as moving average over three consecutive sampling periods.

mals with a single EAR, both at 6 hours and at 24 hours after allergen provocation. In contrast, the dual responders showed a significant hyperreactivity at 6 hours ($p < 0.05$), as well as at 24 hours ($p < 0.001$). In the continuous dosage protocol dual responders also showed a significant increase in reactivity, both at 6 hours ($p < 0.05$) and at 24 hours ($p < 0.05$). No significant differences in BHR were observed between the two provocation protocols. For both protocols, the BHR at 6 hours

was significantly greater than at 24 hours ($p < 0.01$ for the increasing dosage provocation protocol and $p < 0.05$ for the continuous provocation protocol). Linear regression analysis of all data demonstrated a positive correlation between the PC_{100} ratio observed at 6 hours and that determined at 24 hours ($r = 0.43$, $p = 0.006$). Surprisingly, in the two animals with only a single LAR, no apparent BHR was observed, both before and after the LAR (Table II).

TABLE II. Bronchial responsiveness to histamine in guinea pigs after saline or allergen provocation

Provocation procedure	n	Ratio PC ₁₀₀ (before/after challenge)	
		6 hr	24 hr
Saline provocation	5	1.11 ± 0.22	0.84 ± 0.08
Allergen provocation			
Increasing dosages			
Single EAR	6	1.72 ± 0.59	0.97 ± 0.07
EAR + LAR	15	3.67 ± 0.62*	1.65 ± 0.10*
Continuous dosage			
Single EAR	1	2.04	0.97
EAR + LAR	10	2.65 ± 0.42†	1.62 ± 0.19‡
Single LAR	2	0.96; 1.32	0.96; 1.20

Data represent mean values ± SEM. Statistical analysis was done with paired Student's *t* test.

**p* < 0.001.

†*p* < 0.01.

‡*p* < 0.05.

TABLE III. Total and differential leukocyte counts in BAL from unchallenged, saline-challenged, and allergen-challenged guinea pigs

Procedure	Total cells (× 10 ⁶)	Eosinophils (× 10 ⁶)	Neutrophils (× 10 ⁶)	Lymphocytes (× 10 ⁶)	Macrophages (× 10 ⁶)
Unchallenged	22.60 ± 2.87	1.65 ± 0.45	0.08 ± 0.03	1.12 ± 0.46	19.50 ± 2.21
6 hr after					
Saline	17.47 ± 1.59	1.59 ± 0.16	0.07 ± 0.01	0.68 ± 0.20	15.04 ± 1.37
Allergen (incr)	22.48 ± 7.88	5.34 ± 1.71	0.98 ± 0.22*	0.23 ± 0.06	15.88 ± 6.83
Allergen (cont)	29.93 ± 5.99	6.69 ± 2.94	3.10 ± 0.94†	0.39 ± 0.05	19.54 ± 4.56
24 hr after					
Saline	20.18 ± 1.85	1.07 ± 0.18	0.11 ± 0.04	0.85 ± 0.20	17.70 ± 1.86
Allergen (incr)	28.33 ± 1.82*	8.17 ± 0.83‡	3.39 ± 0.85†	0.53 ± 0.12	16.19 ± 1.19
Allergen (cont)	29.95 ± 3.60†	8.90 ± 2.58†	1.94 ± 0.47†	0.58 ± 0.16	18.49 ± 1.09

Data represent mean values ± SEM for five to nine animals. Statistical analysis was done with unpaired Student's *t* test compared with appropriate saline control.

incr, Increasing dosages provocation protocol; *cont*, continuous dosage provocation protocol.

**p* < 0.01.

†*p* < 0.05.

‡*p* < 0.001.

When all data were taken together, there was a significant correlation between the maximal increase in P_{pl} of the EAR and the PC₁₀₀ ratios observed both at 6 hours and at 24 hours after antigen provocation ($r = 0.36$; $p = 0.02$ and $r = 0.45$; $p = 0.004$, respectively). In addition, the AUC of the EAR was significantly correlated with the BHR at 24 hours ($r = 0.35$, $p = 0.03$) but not at 6 hours ($r = 0.23$, $p = 0.17$). The AUC of the LAR was not significantly correlated to the BHR at 6 hours ($r = 0.08$; $p = 0.64$) or at 24 hours ($r = 0.28$; $p = 0.08$).

BAL

Total and differential leukocyte counts recovered in the BAL from unchallenged, saline-chal-

lenged, and allergen-challenged guinea pigs are presented in Table III. In all groups the recovery of the lavage fluid exceeded 80%, with an overall average of $85.0\% \pm 0.7\%$ ($n = 42$). The viability of the recovered cells averaged $94.7\% \pm 0.4\%$. Total cell number and differential leukocyte counts in the BAL were not significantly changed at 6 hours and 24 hours after a saline challenge when compared with those for unchallenged guinea pigs (Table III). When compared with the saline control challenge, a threefold increase in the number of eosinophils was found at 6 hours after allergen provocation when the increasing dosage protocol was used, and a fourfold increase was found when the continuous inhalation protocol was used. These changes were, however, not

statistically significant. At this time point, a 14-fold increase in the number of neutrophils was observed when the increasing dosage protocol was used ($p < 0.01$), whereas this increase was 44-fold when the continuous inhalation protocol was used ($p < 0.05$). The difference in neutrophil counts between both provocation protocols was, however, not statistically significant. At 24 hours after ovalbumin challenge, the number of eosinophils further increased to approximately eightfold for both the increasing dosage protocol ($p < 0.001$) and the continuous inhalation protocol ($p < 0.05$), whereas the number of neutrophils remained at 6-hour levels for both allergen inhalation protocols ($p < 0.05$ for both).

The influx of eosinophils and neutrophils caused an increase in total cell numbers, which was significantly different from control at 24 hours after provocation ($p < 0.01$ and $p < 0.05$ for the increasing dosage protocol and the continuous inhalation protocol, respectively). Total numbers of lymphocytes and macrophages did not change significantly from appropriate control values in both allergen administration protocols.

In a number of experiments BAL cell counts were performed with documented bronchial obstructive reactions and BHR. No significant correlation was found between the number of eosinophils or neutrophils recovered at 6 hours after provocation and the severity of the preceding EAR, determined by the initial decrease and by the AUC ($n = 6$), or the BHR at 6 hours ($n = 11$). In addition, there was no significant correlation between the BAL counts at 24 hours after provocation and the severity of the preceding EAR, LAR or BHR at 6 hours or 24 hours after provocation in a group of nine dual responding animals.

DISCUSSION

Various guinea pig models have been developed to investigate mechanisms involved in the pathogenesis of allergic asthma. In these models major characteristics of this disease including allergen-induced early- and late-phase obstructive reactions, bronchial hyperresponsiveness, and airway inflammation have been observed. However, the relationships among these characteristics within a single model have thus far not been established, whereas the results obtained with the different models have been highly variable.

There are only few reports of allergen-induced late asthmatic responses,^{9-11, 25, 26} with a large variation in frequency, severity, duration, and time of onset after allergen provocation. These differences

are possibly related to variations in sensitization procedures and the subsequent development of IgE homocytotropic antibodies,^{9, 11, 25} differences in challenge procedures,^{9, 25, 26} and the use of histamine H₁-receptor antagonists to prevent anaphylactic shock.^{10, 11, 26} In addition, the variability in onset and duration of the LAR may cause an inadequate description of this reaction by conventional methods of airway function measurement, when anesthesia or body plethysmography is used, because these methods are not suitable for continuous measurement of airway function during prolonged periods.

To this end, we recently developed a new method to assess airway function in chronically instrumented, conscious, and unrestrained guinea pigs.²² In this IgE-sensitized guinea pig model, we observed the development of an LAR in approximately 70% of the animals after allergen provocation, without prophylactic administration of antihistamines. This frequency is comparable to that of most studies in patients with asthma.²⁷

Marked oscillations in P_{pl} were observed during the LAR, which emphasizes the necessity for continuous registration of this response, because only one or just a few airway function measurements during the LAR may result in failure to recognize this response. The cause of these observed oscillations in P_{pl} is presently unknown. One explanation for these fluctuations could be the clearance of mucous plugs by cough during the LAR. The oscillations are not related to the provocation protocols used in this study (i.e., repeated inhalations of stepwise increasing concentrations of the allergen) or to inhalation of one concentration of the allergen during an increasing length of time. Nor were there any differences in onset and severity of the LAR between the two allergen provocation regimens.

At present, it is still unclear why some patients with asthma have a single early response, whereas others are dual responders. As in studies of human asthma,²⁷ we found a close association between the maximal airway obstruction of the EAR and the severity of the LAR, indicating that early events during the EAR may determine the development of this reaction. The most consistent finding in human beings who are dual asthmatic responders is the high level of circulating allergen-specific IgE antibodies, as determined by RAST.²⁷ Because the degree of allergen sensitization determines the quantity of chemical mediators released from activated mast cells, and perhaps other cells, on challenge with a given dose of allergen, these findings suggest that the occur-

rence of a late response is crucially dependent on the quantity of mediators released in the airways.

In guinea pig models BHR has thus far only been investigated in animals with (occasionally documented) early responses at various time points after allergen provocation.¹⁵⁻¹⁹ These studies indicated that BHR may develop as early as 1 hour after the challenge¹⁹ and may last for up to 5 days.¹⁷ Again, marked differences were found in the degree and duration of the BHR, which may be attributed to differences in sensitization procedure¹⁶ and in the nature and route of administration (intravenously or by inhalation) of the bronchoconstrictor used.¹⁷

In our study when inhaled histamine was used to assess BHR, both the early (6-hour) and late (24-hour) changes in bronchial reactivity appeared to be critically dependent on the occurrence of dual asthmatic reactions. This observation corresponds closely with a number of studies of human asthma. Thus initial studies by Cockcroft et al.¹ and Cartier et al.² demonstrated that late changes in BHR only developed in patients with a definite LAR. More recent studies demonstrated that BHR may already be present just after the EAR,^{3, 6, 7, 28} but as in our study, predominantly in subjects with a dual response.^{3, 7, 28} In addition, in some of these studies^{3, 7} a significant correlation was found between the BHR after the EAR and the severity of the LAR, suggesting that the early developed BHR may contribute to this response. However, such a significant correlation was not found in a recent study by Bernstein et al.,²⁸ as well as in our study in guinea pigs. Furthermore, our observation that a single LAR may develop without prior development of BHR (Table II) argues against a direct causal relationship between the early development of BHR and the LAR. Remarkably, we were also unable to detect BHR after the single late asthmatic responses, which would suggest that the BHR at 24 hours after allergen provocation is not associated with the LAR. As in some studies of human asthma,^{3, 28, 29} this was confirmed by the absence of a significant correlation between the severity of the LAR and the degree of BHR after the LAR in the whole population. In contrast, when all data were taken together, significant correlations were found between the maximal increase in P_{pl} of the EAR and the BHR at both 6 hours and 24 hours after provocation, indicating that early events during the EAR may determine both early and late changes in BHR. A close correlation was observed between the BHR at 6

hours and the (reduced) BHR at 24 hours. This may suggest that the late BHR is merely a reduction of the early BHR and not, as discussed above, a feature of the LAR. Evidence for a reduced BHR at 24 hours after provocation, compared with early changes in BHR, was also obtained in some studies of human asthma.^{7, 28}

The mechanism of the observed allergen-induced early and late changes in bronchial reactivity is presently unknown. The BHR could in part be related to geometric changes in the airways caused by thickening of the mucosa and the presence of secretions in the airway lumen.³⁰ It should, however, be noted that BHR was assessed between the EAR and LAR, as well as after the LAR, when P_{pl} was at or near the baseline value, indicating that a reduced airway caliber did not contribute to the observed BHR.

Alternatively, BHR could result from a number of changes in the neurogenic or humoral control of airway smooth muscle or in the airway smooth muscle itself or from damage of the airway epithelium.³¹⁻³³

Because numerous studies have indicated a close association between airway inflammation and BHR in patients with asthma, it is widely assumed that inflammatory cells and their mediators play an important role in the enhanced bronchial responsiveness after allergen challenge.²⁷ Similar to previous observations in guinea pigs,^{10, 11, 16} we found a tendency toward an enhanced number of eosinophils at 6 hours after provocation, whereas the neutrophil count was significantly enhanced at this time point. At 24 hours after provocation, the numbers of both eosinophils and neutrophils were significantly enhanced. In addition, separate histopathologic examinations during the LAR showed a marked infiltration of predominantly eosinophils within the bronchial and bronchiolar walls, without any definite cellular infiltration or thickening of the alveolar septa, indicating that alveolitis is not involved in the observed airway responses.

The time course of BAL eosinophilia correlates closely with that found by De Monchy et al.⁴ in patients with asthma who showed no significant eosinophilia in the BAL at 2 hours after the EAR, whereas the eosinophil count was enhanced during the LAR of dual asthmatic responders. In addition, Metzger et al.⁵ showed a significant neutrophilia at 4 hours and a significant eosinophilia at this time point and at 24 hours after provocation. Moreover, in both studies the eosinophils appeared to be activated, as indicated by signs of degranulation⁵ and enhanced levels of

eosinophil cationic protein in the BAL fluid during the LAR.⁴ The release of granule products including eosinophil cationic protein and major basic protein may cause epithelial damage,^{34, 35} which could contribute to allergen-induced BHR by exposing sensory nerve endings³⁶ or by removing the protective effects of an epithelium-derived relaxant factor.³⁷ Indeed, in patients with mild asthma a significant inverse correlation was found between the provocative concentration causing a 20% decrease in FEV₁ for methacholine and the percentage of eosinophils, the percentage epithelial cells, and the concentration of major basic protein in the BAL.³⁸

However, in our study we found a dissociation between the time course of eosinophil infiltration in the airways, being only significant and maximal at 24 hours after provocation, and BHR, being maximal at 6 hours and decreased at 24 hours after provocation. In addition, we found no significant correlation between the eosinophil counts in the BAL and BHR, both at 6 hours and 24 hours after provocation. Absence of a correlation between allergen-induced airway eosinophil accumulation and BHR at various time points after provocation was also found by Sanjar et al.¹⁶

One explanation for this dissociation could be a marked difference between time course of tissue eosinophilia, which may occur very rapidly (within 10 minutes) after allergen provocation, and the appearance of these cells in the BAL, occurring between 6 and 18 hours after provocation.¹⁴ Another contributing factor could be a dissociation between the number of infiltrated eosinophils and the activation state of these cells, which will be relevant to the development of BHR. Recently, such a dissociation was indeed found in a study by Aalbers et al.³⁹ in patients with asthma. In this study it was found that the number of eosinophils in the BAL was not significantly enhanced after the EAR at 3 hours after allergen provocation, whereas the number of activated eosinophils, as indicated by immunocytochemical staining for the activated form of eosinophil cationic protein (EG2-positive cells), was significantly enhanced at this time point. In addition, this number was significantly enhanced in patients with a dual response compared with patients with a single EAR, indicating that the activation of these cells may indeed be related to the early development of BHR.

In conclusion, in a new animal model of allergic asthma, using chronically instrumented, conscious, and unrestrained guinea pigs, we found relation-

ships between allergen-induced early- and late-phase asthmatic reactions, early and late changes in bronchial reactivity and airway inflammation, which were strikingly similar to those observed in human asthma. Therefore we consider this model to be a useful tool for further detailed investigations into the pathogenesis of allergic asthma and bronchial hyperresponsiveness.

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