Characterization of allergen-induced bronchial hyperresponsiveness and airway inflammation in actively sensitized Brown-Norway rats

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Bronchial responsiveness to inhaled acetylcholine (ACh) and inflammatory cell recruitment in bronchoalveolar lavage fluid (BALF) were studied in inbred Brown-Norway rats actively sensitized to, and later exposed to, ovalbumin (OA). We examined animals 21 days after initial sensitization at 18 to 24 hours, or 5 days after a single challenge, or after the last of seven repeated exposures administered every 3 days. BALF was examined as an index of inflammatory changes within the lung. Animals repeatedly exposed to OA aerosols had an increased baseline lung resistance and a significant increase in bronchial responsiveness to inhaled ACh compared to control animals at both 18 to 24 hours and 5 days after the last OA exposure. Sensitized animals receiving a single OA aerosol also demonstrated bronchial hyperresponsiveness (BHR) to inhaled ACh ($p < 0.01$) at 18 to 24 hours of a similar order as the multiple-exposed group. There was a significant increase in eosinophils, lymphocytes, and neutrophils in BALF at 18 to 24 hours but not at 5 days after single or multiple exposure to OA aerosol in the sensitized groups. Control animals demonstrated no changes in bronchial responsiveness, although a small but significant increase in inflammatory cells was observed compared to saline-only treated animals. There was a significant correlation between bronchial responsiveness and eosinophil counts in the BALF in the single allergen-exposed group ($R_s = 0.68$; $p < 0.05$). We conclude that (1) BHR after allergen exposure in sensitized rats is associated with the presence of pulmonary inflammation but persists despite the regression of inflammatory cells in BALF after multiple OA exposures, and (2) this rat model has many characteristics of human allergen-induced BHR. (J ALLERGY CLIN IMMUNOL 1991;88:951-60.)

Key words: *Brown-Norway rats, bronchial hyperresponsiveness, active sensitization, ovalbumin, bronchoalveolar lavage*

BHR to a wide variety of bronchoconstrictor stimuli is a characteristic feature of asthma[1, 2] and is closely related with the severity and frequency of asthma symptoms.[3] Although the precise mechanism(s) of this phenomenon have not been elucidated, considerable attention has been focused on the role of airway inflammation in the pathophysiology of BHR. Experimental studies in several animal models, including dog, rabbit, and sheep, have demonstrated a close temporal association between the degree of inflammatory cell infiltration in the airway wall and the development of BHR induced by several stimuli, in-

Abbreviations used

ACh:	Acetylcholine
BHR:	Bronchial hyperresponsiveness
BALF:	Bronchoalveolar lavage fluid
OA:	Ovalbumin
PC_{200}:	Provocative concentration producing 200% increase in lung resistance
R_L:	Lung resistance
R_s:	Spearman's rank-correlation coefficient
$Al(OH)_3$:	Aluminium hydroxide
ip:	Intraperitoneal
Ab:	Antibody
EOS:	Eosinophil

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TABLE I. Characteristics of eight experimental groups and procedures performed for each group

Group	N	Body weight (g)	ip Injection (× 3)	Aerosol exposure	Baseline R_L (cm H_2O $ml^{-1}s^{-1}$)	Challenge (time after last exposure)
A	9	265 ± 10	Saline	Saline (× 1)	0.44 ± 0.03	18-24 hr
B	8	248 ± 10	$Al(OH)_3$	—	0.46 ± 0.03	—
C	6	255 ± 8	OA plus $Al(OH)_3$	—	0.53 ± 0.01	—
D	5	243 ± 12	—	OA (× 1)	0.51 ± 0.03	18-24 hr
E	11	240 ± 14	OA plus $Al(OH)_3$	OA (× 1)	0.49 ± 0.05	18-24 hr
F	8	253 ± 11	OA plus $Al(OH)_3$	OA (× 7)	0.60 ± 0.05*	18-24 hr
G	5	260 ± 18	OA plus $Al(OH)_3$	OA (× 7)	0.53 ± 0.06	5 days
H	6	257 ± 14	OA plus $Al(OH)_3$	OA (× 1)	0.43 ± 0.03	5 days

*$p < 0.01$ compared to saline-exposed group.

cluding allergen and ozone.[4] In the sensitized dog, rabbit, and sheep,[5, 6] allergen exposure causes an increase in bronchial responsiveness associated with a neutrophilia in BALF. Allergen challenge in sensitized guinea pigs[7, 8] and monkeys,[9, 10] however, produces an EOS influx into the airways, and these animals have been proposed as better models for human asthma because the EOS, rather than the neutrophil, appears to be a more prominent cell in the airway submucosa of patients with asthma. Similar studies have been performed in sensitized rats that are known to develop specific Abs of the IgE class,[11, 12] in contrast to guinea pigs that usually demonstrate the presence of IgG Abs.[13] After allergen exposure, these rats develop both an acute and a late bronchoconstrictor response,[12] and increases in bronchial responsiveness to methacholine[14] similar to the responses observed in human subjects with asthma.[15]

We have characterized further this model by studying the effect of allergen challenge on bronchial responsiveness and on the influx of cells into the lungs and airways with BAL. We examined the duration of the induced BHR and determined whether repeated exposure to aerosolized allergen compared to a single allergen exposure could further enhance and prolong this BHR and its accompanying inflammatory response. In addition, we examined the effects of sensitization per se, of treatment with adjuvant only and of exposure of unsensitized animals to OA aerosol on bronchial responsiveness and cellular composition of BALF.

METHODS

Sensitization procedures and allergen exposure

We studied eight separate groups of inbred male Brown-Norway rats weighing 200 to 350 g. Seven of the eight groups were administered ip injections on days 1, 2, and 3. The object of the eight groups used was (1) to determine the effects of a single or multiple exposure to allergen after ip sensitization, (2) to compare the time course after the last allergen exposure between 18 to 24 hours and 5 days in the single- and multiple-exposure groups, (3) to determine the effect of ip sensitization per se and of adjuvant alone, and (4) to examine the effect of aerosolized allergen exposure in unsensitized animals. The groups are summarized in Table I and were as follows:

Group A (saline). One milliliter of saline was injected intraperitoneally, followed 21 days later by a single saline-aerosol exposure for 15 minutes. Animals were studied 18 to 24 hours later.

Group B ($Al(OH)_3$ only). One milliliter of a 100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally but no aerosol exposure.

Group C (sensitized; no allergen aerosol exposure). One milliliter of a 1 mg OA/100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally; this group was not exposed to allergen.

Group D (allergen aerosol exposure only). Naive animals were exposed to a 1% OA aerosol for 15 minutes; animals were studied 18 to 24 hours later.

Group E (single allergen aerosol exposure). One milliliter of a 1 mg OA/100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally, followed 21 days later by a single exposure to 1% OA aerosol for 15 minutes; animals were studied 18 to 24 hours later.

Group F (chronic allergen aerosol exposure). One milliliter of a 1 mg OA/100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally, followed by exposure to 1% OA aerosol for 15 minutes administered every third day for a 21-day period; animals were studied 18 to 24 hours after their last exposure.

Group G (5 days after chronic allergen aerosol exposure). One milliliter of a 1 mg OA/100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally, followed by exposure to 1% OA aerosol for 15 minutes administered every third day for a 21-day period; animals were studied 5 days after their last exposure.

Group H (5 days after single allergen aerosol exposure). One milliliter of a 1 mg OA/100 mg $Al(OH)_3$ in 0.9% (wt/vol) saline suspension was injected intraperitoneally,

followed 21 days later by a single exposure to 1% OA aerosol for 15 minutes; animals were studied 5 days later.

Aerosol exposure was accomplished by placing the rats in a 6.5 liter plexiglass chamber connected to a DeVilbiss PulmoSonic nebulizer (model No. 2512, DeVilbiss Health Care, U.K., Ltd., Feltham, Middlesex, U.K.) that generated an aerosol mist pumped into the exposure chamber by the airflow supplied by a small animal ventilator set at 60 strokes/min^{-1} with a pumping volume of 10 ml.

Measurement of lung function and airway responsiveness to ACh

For measurement of airway responsiveness, rats were anesthetized with an initial dose of 60 to 80 mg/kg of pentobarbitone injected intraperitoneally. Additional pentobarbitone was administered as required to maintain adequate anesthesia. A tracheal cannula (10 mm length and 1.3 mm internal diameter) was inserted into the lumen of the cervical trachea through a tracheostomy and tied snugly with suture material. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure and heart rate with a pressure transducer. The right external jugular vein was cannulated for administration of intravenous drugs and fluids. Animals were then connected to a small animal respirator (Harvard Apparatus, Ltd., Edenbridge, Kent, U.K.) and ventilated with 10 ml/kg^{-1} air at a rate of 90 strokes/min^{-1}. Transpulmonary pressure was measured with a pressure transducer (model FCO 40; ± 1000 mm of H_2O, Furness Controls, Ltd., Bexhill, Sussex, U.K.) with one side attached to an air-filled catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. The ventilatory circuit had a total volume of 20 ml. Airflow was measured with a pneumotachograph (model F1L; Mercury Electronics, Ltd., Glasgow, Scotland) connected to a transducer (model FCO 40; ± 20 mm of H_2O, Furness Controls, Ltd.). The signals from the transducers were digitalized with a 12-bit analog-digital board (NB-MIO-16, National Instruments, Austin, Texas) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, Calif.) and analyzed with a software (LabView, National Instruments), which was programmed to instantaneously calculate R_L according to the method of von Neergard and Wirz.[16] Transpulmonary pressure and mean blood pressure were also monitored throughout the experiments. Aerosols were generated with an ultrasonic nebulizer (model 2511; PulmoSonic, DeVilbiss Co., Pa.) and were administered to the airways through a separate ventilatory system that bypassed the pneumotachograph. The volume of this circuit was 50 ml. The mean mass diameter of the aerosol was 3.8 μm, with a geometric standard deviation of 1.3, measured with a laser droplet and particle analyzer (model 2600C, Malvern Instruments, Derbyshire, U.K.).

Animals were initially injected with propranolol (1 mg/kg intravenously) to inhibit adrenergic effects. A dose of inhaled saline was administered for 45 breaths, and the subsequent R_L value was used as baseline. Starting 3 minutes after saline exposure, increasing half-$\log_{10}$ concentrations of ACh were administered by inhalation (45 breaths) with the initial concentration set at 10^{-4} mol/L. Increasing concentrations were administered at 5- to 7-minute intervals with one hyperinflation of twice the tidal volume applied between each ACh concentration, performed by manually blocking the outflow of the ventilator. The challenge was stopped when an increase in R_L exceeding 200% over the initial baseline was obtained. PC_{200} was calculated by log-linear interpolation of concentration-response curves from individual animals.

BAL and cell counting

After measurement of lung-function parameters, rats were administered an overdose of sodium pentobarbitone (100 mg/kg^{-1} intravenously), and lungs were lavaged with 10 × 2 ml aliquots of 0.9% wt/vol of sterile saline through a polyethylene tube introduced through the tracheostomy. Lavage fluid was centrifuged (500 *g* for 10 minutes at 4° C), and the cell pellet was resuspended in 0.5 ml of Hanks' balanced salt solution. Total cell counts were made by adding 10 μl of the cell suspension to 90 μl of Kimura stain and counted in a Neubauer (American Optical Corp., Southbridge, Mass.) chamber under a light microscope. Differential cell counts were made from cytospin preparations stained by May-Grünwald stain. Cells were identified as EOSs, lymphocytes, neutrophils, and macrophages by standard morphology and 500 cells counted under ×400 magnification, and the percentage and absolute number of each cell type were calculated.

Drugs

We used the following drugs: OA (grade V, salt free) and ACh (Sigma Chemical Co., Poole, Dorset, U.K.), aluminum hydroxide (BDH Chemicals Ltd., Dorset, Poole, U.K.), propranolol (Inderal, ICI, plc, Macclesfield, Cheshire, U.K.), and pentobarbitone sodium (Sagatal, May & Baker, Ltd., Dagenham, U.K.).

Data analysis

PC_{200} data have been $\log_{10}$ transformed and are reported as mean $-\log PC_{200}$ ± SEM. Nonparametric analysis of variance (Kruskal-Wallis method) was used to determine significant variance among the eight groups. If a significant variance was found, we used Mann-Whitney U test to analyze for significant difference between individual groups, and since more than one comparison was made, a p value of <0.01 was considered significant. Spearman rank-correlation coefficient (Rs) was used to analyze the relationship between variables. Data were analyzed with a Macintosh computer (Apple Computer Inc.) with standard statistical packages.

RESULTS

Baseline analysis

Baseline R_L and body weight are presented for each individual group in Table I. A single allergen exposure (group E) did not significantly increase the baseline R_L 18 to 24 hours later compared to the saline-exposed

group (group A). However, after repeated allergen exposure (group F), baseline R_L was significantly higher than after saline exposure only ($p < 0.01$) (Table I).

Aerosol exposure

Animals sensitized by ip injection of 1 ml of 1 mg OA/100 mg $Al(OH)_3$ responded when they were exposed to an aerosol of 1% OA solution by demonstrating obvious respiratory distress characterized by the development of a defensive posture and exaggerated labored breathing motions. This immediate response started within 3 to 5 minutes of aerosol administration, but its appearance did not result in the death of any animal nor did it necessitate the use of protective drug cover. All animals recovered spontaneously from aerosol exposure. Animals administered saline intraperitoneally and then exposed to a subsequent saline aerosol did not demonstrate this immediate response, nor did unsensitized animals exposed to aerosolized allergen.

Airway responsiveness

Inhaled ACh caused a concentration-dependent increase in R_L. Individual peak R_L responses at each challenge concentration for animals in groups A, E, and F are illustrated in Fig. 1. After inhalation of each concentration of ACh, peak R_L was reached within 45 seconds and recovered during 2 to 5 minutes. Mean $-\log PC_{200}$ values for all the groups are also illustrated in Fig. 2.

The airway responsiveness of the three groups that received the adjuvant $Al(OH)_3$ only (group B), OA with $Al(OH)_3$ without a subsequent allergen aerosol exposure (group C), and the group that only received an allergen aerosol exposure (group D) demonstrated a small but nonsignificant change in responsiveness compared to saline-only treated animals (group A) that had a $-\log PC_{200}$ value of 1.8 ± 0.12 mol/L of ACh.

The two groups that had been intraperitoneally sensitized and then exposed to either a single or a multiple allergen challenge and tested 18 to 24 hours after the last exposure (groups E and F) were significantly more responsive ($p < 0.01$) than the four control groups. Hyperresponsiveness to ACh was also present when the chronic allergen-exposed rats were tested 5 days after their last aerosol exposure (group G) with a mean $-\log PC_{200}$ value of 2.6 ± 0.12 mol/L of ACh ($p < 0.01$). The group of sensitized animals administered a single allergen exposure and tested 5 days later (group H) was not significantly more responsive than the control group A.

The greatest increase in responsiveness was observed in the chronic allergen-exposed group (group F) with a $-\log PC_{200}$ value of 2.9 ± 0.19 mol/L of ACh, which is approximately a tenfold increase in airway responsiveness compared to the saline-treated group ($p < 0.01$). However, the single allergen aerosol-exposed rats (group E) produced a similar increase in airway responsiveness to those chronically exposed, revealing a $-\log PC_{200}$ value of 2.6 ± 0.11 mol/L of ACh.

Cellular content of BALF

BALF recovered from sensitized animals that had been exposed to a 1% OA aerosol had significantly increased total cell numbers 18 to 24 hours later (group E, $4.33 \pm 0.55 \times 10^6$; group F, $4.29 \pm 0.92 \times 10^6$) ($p < 0.01$) compared both to saline-treated animals (group A, $2.18 \pm 0.39 \times 10^6$) or to animals not exposed to allergen aerosol (group B, $1.92 \pm 0.34 \times 10^6$; group C, $1.47 \pm 0.10 \times 10^6$) or to unsensitized animals exposed to 1% OA (group D, $1.91 \pm 0.48 \times 10^6$) (Fig. 3).

There was a significant preferential increase in the numbers of EOSs and lymphocytes in groups E and F when these groups were compared to all the other groups ($p < 0.01$) (Fig. 4): saline-treated animals (group A: EOSs, $0.013 \pm 0.01 \times 10^6$; lymphocytes, $0.01 \pm 0.02 \times 10^6$), single allergen-exposed animals (group E: EOSs, $1.34 \pm 0.31 \times 10^6$; lymphocytes, $0.57 \pm 0.12 \times 10^6$), and chronic allergen-exposed animals (group F: EOSs, $1.42 \pm 0.35 \times 10^6$; lymphocytes, $0.73 \pm 0.32 \times 10^6$). Total BAL cell counts from groups G and H were not significantly different from counts from control groups B, C, and D. The EOS and lymphocyte counts from groups G and H were significantly lower than counts from groups E and F, respectively. However, the animals from the chronically exposed group tested 5 days later (group G) were still hyperresponsive. There was a small, but significant, increase in inflammatory cell infiltration (i.e., EOSs, lymphocytes, and neutrophils) in animals from groups B, C, and D ($p < 0.01$) when compared to saline-only treated animals.

Relationship between BHR and cells in BALF

There was a significant correlation between bronchial responsiveness (PC_{200}) and the degree of EOS, lymphocyte, and neutrophil infiltration when these data from all the different experimental groups were analyzed together (for EOSs, $R_s = 0.78$; $p < 0.01$; for lymphocytes, $R_s = 0.36$; $p < 0.02$; for neutrophils, $R_s = 0.48$; $p < 0.01$). When the sensitized, single allergen-exposed group (group E) was analyzed alone, this significant relationship remained only between EOS count and the PC_{200} to ACh ($R_s = 0.68$; $p < 0.05$; Fig. 5).

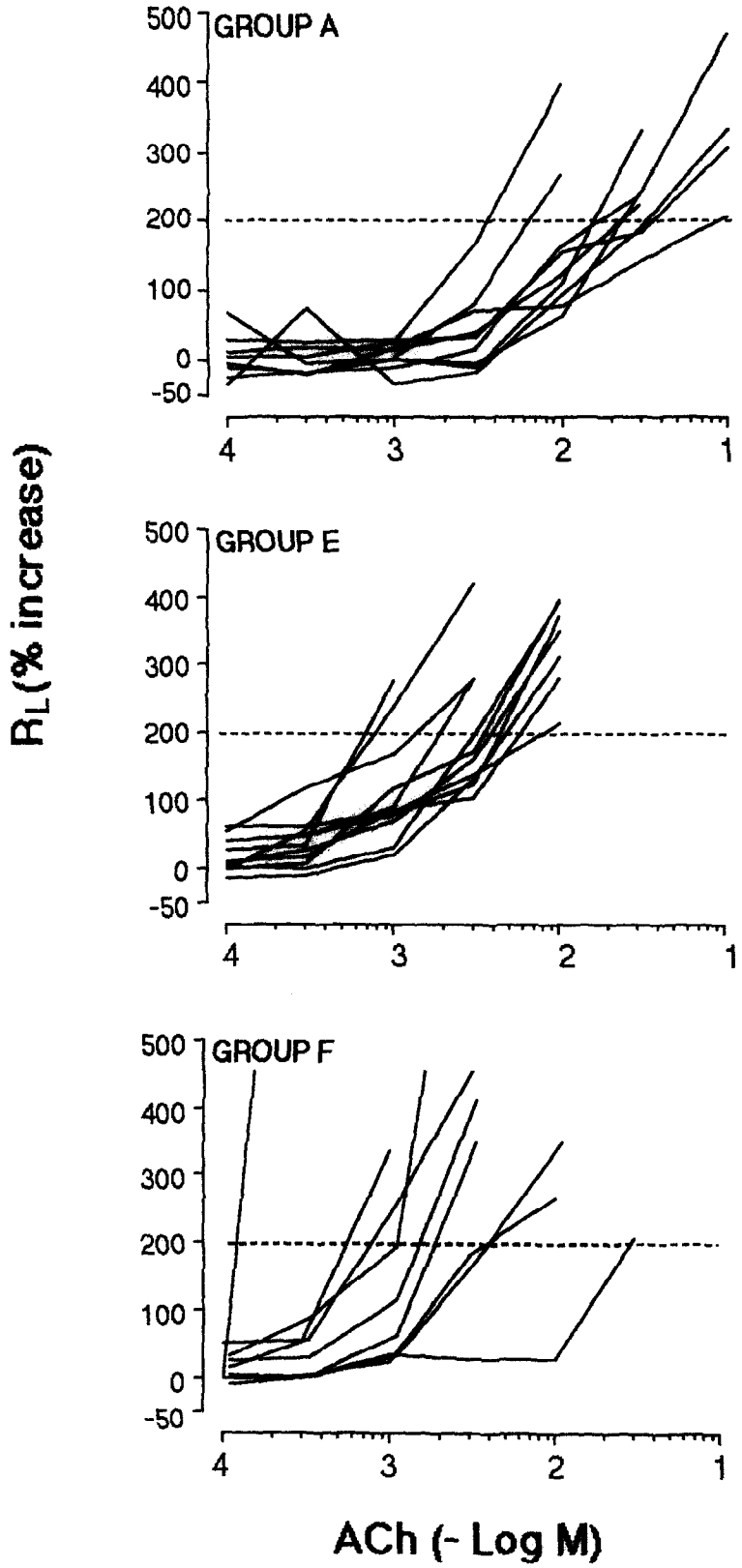

FIG. 1. Individual concentration R_L relationships for inhaled ACh for the individual rats from group A (nonsensitized, saline exposed), group E (sensitized, single allergen exposed), and group F (sensitized, chronic allergen exposed). R_L is expressed as percent increase above baseline. The *horizontal dotted line* represents the 200% increase above baseline and its intersection with the concentration-response curve and indicates the individual PC_{200} values.

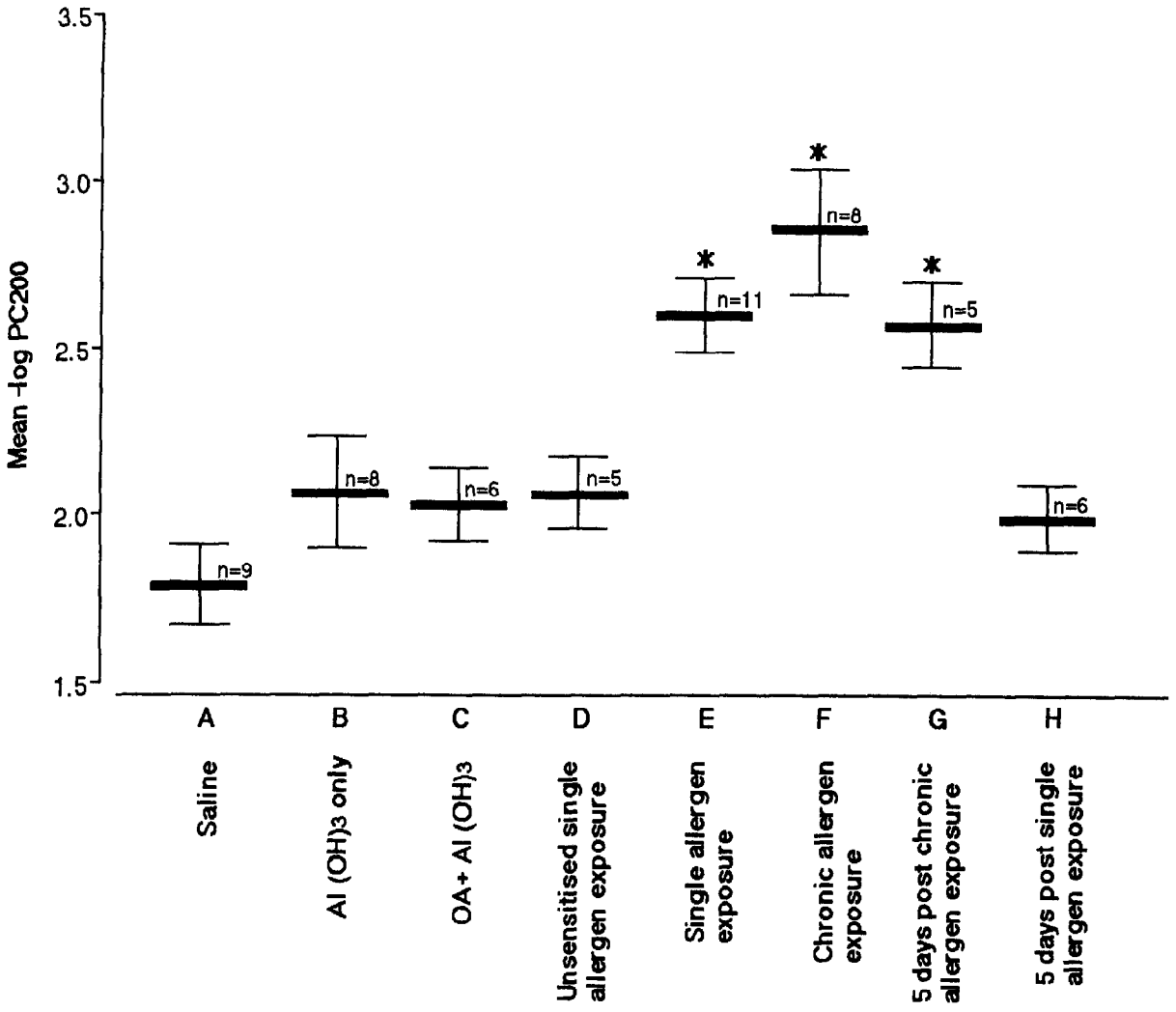

FIG. 2. Mean $-\log PC_{200}$ ($\pm$ SEM) for groups A to H (see Table I for the individual groups). Only groups E, F, and G reveal a significant increase in bronchial responsiveness compared to that of control group A ($*p < 0.01$).

DISCUSSION

In this in vivo rat model, we have demonstrated that bronchial responsiveness to inhaled ACh was significantly increased at 18 to 24 hours in intraperitoneally sensitized rats after exposure to OA aerosol compared to bronchial responsiveness of unsensitized or unexposed groups. The increase in responsiveness observed was of a similar order of sevenfold to tenfold in rats exposed either to a single allergen aerosol or to multiple aerosols. BHR observed after a single OA aerosol was detectable at 18 to 24 hours after exposure but was not present 5 days later. However, in animals exposed repeatedly to OA aerosols, there was a more persistent BHR that was also observed 5 days after the last exposure. In this respect, our results are similar to results of Eidelman et al.[12] who were unable to demonstrate a change in airway responsiveness to inhaled methacholine in sensitized rats, despite the development of an identifiable late response after a single exposure to OA aerosol measured 1 week after a single exposure. Bellofiore and Martin[14] also demonstrated an increase in methacholine responsiveness in sensitized rats after multiple OA aerosol exposures lasting for up to 17 days after the last challenge.

Brown-Norway rats are good producers of IgE Ab and almost uniformly respond to sensitization with the development of increased titers of specific IgE Ab.[17] As an index of the allergic inflammatory changes within the lungs, we used cell counts obtained by BAL. We demonstrated an increase in the total cells recovered in all groups that were sensitized and then exposed to aerosolized allergen. The biggest increases in EOS, lymphocyte, and neutrophil counts occurred 18 to 24 hours after allergen exposure in the sensitized

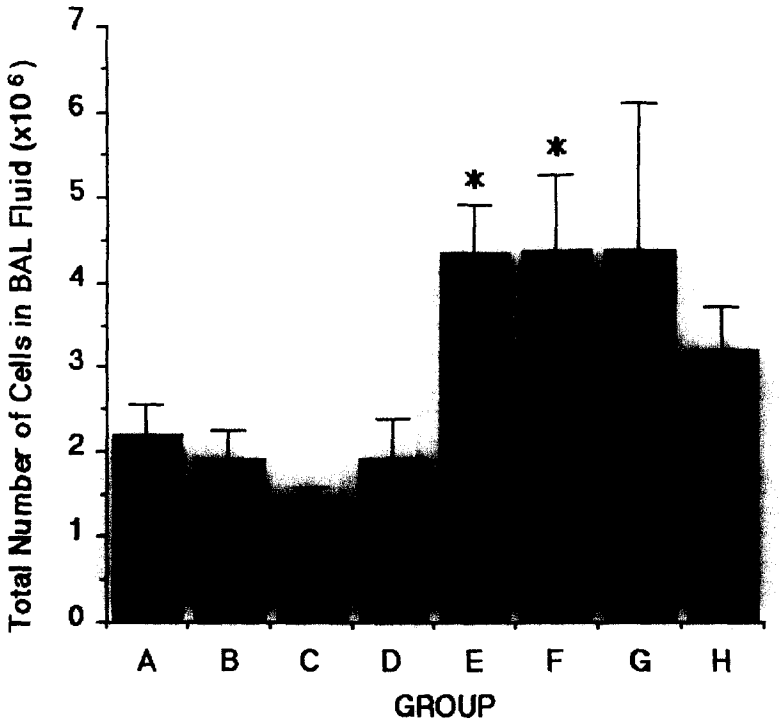

FIG. 3. Mean total cell counts (± SEM) in BALF from groups A to H (see Table I for the individual group treatments). There was a significant increase in total cells in groups E and F compared to that of control groups A to D ($*p < 0.01$).

animals, with a reduction in these cell types after 5 days to levels not significantly different from that of the control groups. A more variable increase in macrophage recovery in BALF by day 5 after the last allergen challenge was observed in the group of rats that demonstrated persistently increased responsiveness to inhaled ACh (group G). Unlike the ragweed sensitized dog model,[5] we found a slight, but nonsignificant, increase in neutrophils in BALF after allergen challenge compared to the intraperitoneally sensitized-only group (group C). The increase in EOS counts that we have demonstrated were transient and were not present 5 days after allergen exposure, unlike the sustained increase in EOS counts recently demonstrated in monkeys sensitized to *Ascaris suum*.[10] Interestingly, our data appear similar to data reported after allergen challenge in human subjects with asthma in whom EOSs were the predominant inflammatory cell type in BALF.[18]

Our studies reveal additional light on the relationship between the inflammatory process induced by allergen challenge and the increase in bronchial responsiveness to inhaled ACh. The BHR observed at 18 to 24 hours after allergen challenge coincided with the largest increases in inflammatory cell types in the BALF. We found a significant relationship between EOS count and PC_{200} when data from all groups were pooled. This significant relationship remained when results from rats in the single OA aerosol-exposed group only were analyzed (group 5). However, in our model, persistence of BHR at 5 days after the last allergen exposure was not accompanied by significant changes in cellular content of BALF. Our data therefore suggest that BHR may persist in the absence of inflammatory cells and that it is possible that the recruitment of inflammatory cells may only be a transient event that initiates mechanisms for BHR that may persist for longer than the inflammatory response itself. BHR that persists for weeks after a single exposure has been described in allergic dogs after allergen challenge, but in this model, no data on the BAL cell content at later time points were obtained.[5] Since we have not examined the histology of the airways in the present study, it is not possible to state whether the profile of cells recovered from the BAL represents a true reflection of submucosal airway inflammation. It is also possible that the persistent BHR that we observed may result from thickening of the airway submucosa from either persistent edema or connective-tissue fibrosis.[19]

We performed control studies in four separate groups of rats to determine whether the adjuvant used, $Al(OH)_3$, or sensitization per se, or exposure of unsensitized rats to OA aerosol had any effect on airway

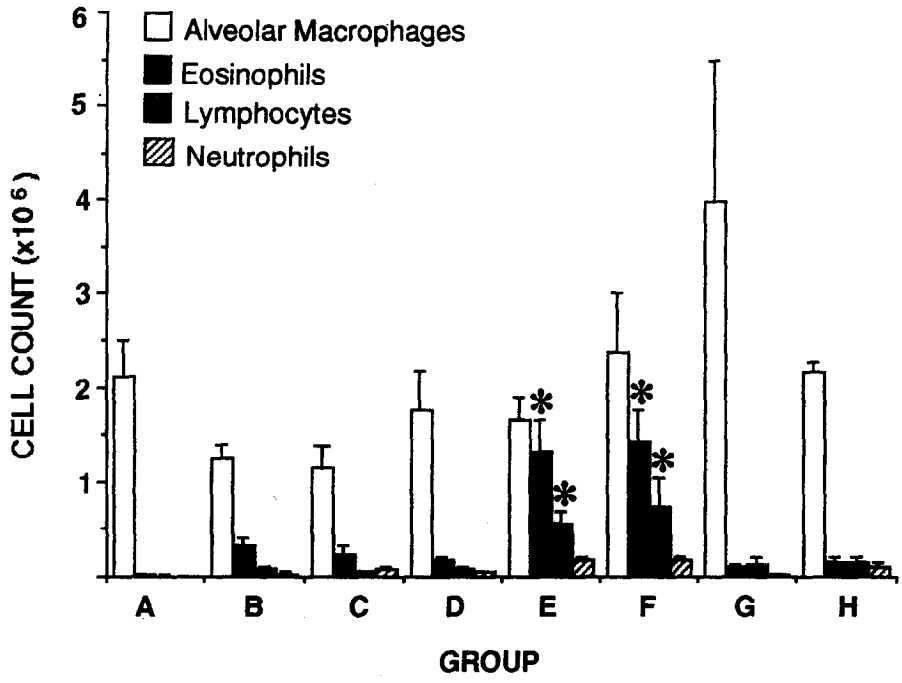

FIG. 4. Mean cell counts (± SEM) for macrophages, EOSs, lymphocytes, and neutrophils (*left* to *right*) from BALF obtained from groups A to H (see Table I for the individual groups). Groups E and F had a significant preferential increase in EOSs and lymphocytes compared to that of all other groups ($*p < 0.01$).

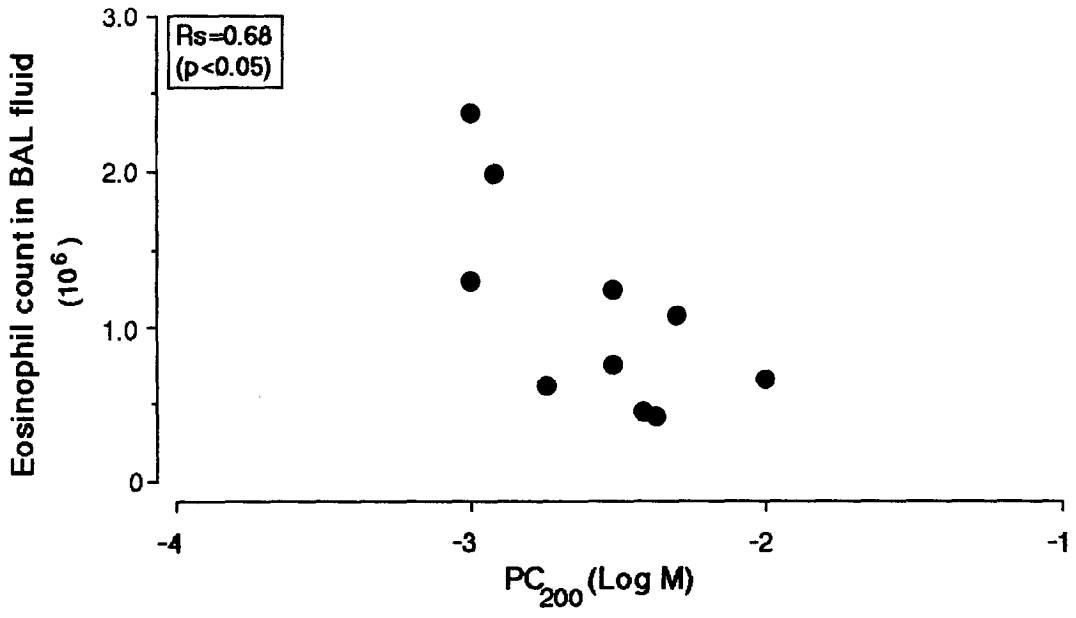

FIG. 5. Relationship between PC_{200} and total number of EOSs in BALF from sensitized, single allergen-exposed animals (group E). There is a significant correlation ($r_s = 0.68$; $p < 0.05$).

responsiveness or inflammatory changes within the lung compared to a saline-only treated group. These procedures caused no significant change in bronchial responsiveness consistent with previous studies in rats, guinea pigs, and dogs,[20-22] and although there were small changes in the relative proportions of cells recovered by BAL, the total cell counts were not affected. These observations suggest that ip sensitiza-

tion itself is not responsible for the large increase in inflammatory cell recovery found in the BALF after allergen exposure and may represent a nonspecific response of the immune system or of the lungs to $Al(OH)_3$ or to aerosolized allergen exposure, respectively. In the saline-only treated group, there were very few EOSs, lymphocytes, or neutrophils, and alveolar macrophages usually accounted for 95% of all recovered cells.

Repeated exposure to OA in sensitized rats did not induce additional increases in airway responsiveness or in total cell recovery compared to those found after a single exposure. This raises the possibility that there may be a process of desensitization to prevent ongoing and increasing inflammation and the attendant BHR, as has been described in guinea pigs sensitized to OA[23, 24] and sheep sensitized to *A. suum*,[25] and as was observed in the rat to some extent by Bellofiore and Martin.[14] The mechanisms for this limitation of BHR and inflammation are not clear, but one possibility is that this could be due to T suppressor cell generation after repeated aerosol challenge that would reduce the amount of chemoattractant lymphokine generation, as has been described in the rat.[26] It is also possible that repeated exposure to OA via the respiratory tract may eventually lead to diminution of IgE titers.[27] Nevertheless, repeated exposure appears to lead to a more persistent increase in airway responsiveness compared to the effect of a single OA exposure.

Of great prominence is the presence of increased numbers of lymphocytes and EOSs in the BALF of the sensitized rats challenged with OA. Current evidence suggests that lymphocytes may be involved in regulating both EOS influx[28] and activation in the airways of patients with asthma[29] through the release of various lymphokines, such as interleukin-5.[30, 31] EOS recruitment and activation in the airways may be an important step in the pathogenesis of BHR.[32] It is also possible that lymphocytes may be important both in enhancing and limiting the degree of BHR in our model, and it is therefore important to investigate further the role of these cells in this animal model of BHR.

In summary, this in vivo model in the sensitized rat demonstrates several features that bear similarities to features of human asthma, including an immediate response after allergen exposure, followed 18 to 24 hours later by a significant increase in EOS and lymphocyte counts in BALF associated with BHR to inhaled ACh after a single allergen exposure, and more sustained BHR after repeated allergen exposure. Our findings in this rat model are therefore of relevance to bronchial asthma in humans, and our model may be used to elucidate the roles of the EOS and lymphocyte in the pathogenesis of human allergic airway inflammatory responses and BHR.

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