

Association of mononuclear cells and eosinophils with airway resistance and responsiveness in rat pulmonary inflammatory responses

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To evaluate the association of various leukocytes with pulmonary resistance and methacholine responsiveness, we induced pulmonary eosinophil-rich inflammation in IgE-sensitized (ovalbumin) Sprague Dawley rats. Sensitized rats were challenged with either relevant (OA) or irrelevant antigen by tracheal insufflation a) with no other treatment, b) in conjunction with intravenous Sephadex beads pretreatment, or c) with antigen coupled covalently to Sepharose beads. About 24 h after antigen challenge, respiratory system resistance (Rrs), response to aerosolized methacholine, and pulmonary histopathology were evaluated. Challenge with OA, insufflation with Sepharose, and treatment with i.v. Sephadex all independently increased inflammatory cell infiltrates, but the combination of OA with the other agents did not significantly enhance the inflammatory response over OA alone. Interactive stepwise regression techniques were utilized to identify correlates for Rrs and methacholine responsiveness. Mononuclear cell score was a significant predictor ($p < .01$) for Rrs, and insufflation of Sepharose had a significant independent effect on Rrs ($p = .01$) above that predicted by mononuclear cell infiltrates. Conversely, eosinophil score and neutrophil score were not significant predictors for Rrs, and challenges with antigen or Sephadex had no significant independent effect on Rrs beyond that predicted from mononuclear cell infiltrates. Eosinophil score was the only significant histological predictor for methacholine responsiveness ($p < .0001$). Challenges with Sephadex, antigen and Sepharose did not significantly change methacholine responsiveness independently of the changes associated with eosinophil infiltrates. These findings suggest that mononuclear cells and eosinophils contribute to increases in airway resistance and responsiveness, respectively, following the induction of pulmonary inflammation by both allergic and non-allergic stimuli.

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Airway obstruction in asthma is now considered to be the result of multiple pathologic factors including airway smooth muscle contraction, increased mucus secretion, edema, and airway inflammation (1). Airway hyperresponsiveness is a major clinical feature of asthma (2), and the contribution of these various pathologic changes to this phenomenon is still unresolved (3). To address these is-

suues, a variety of immunologic and chemical challenge models have been developed (4, 5).

Using a rat model, we have begun to characterize the immunologic (6, 7) and histologic (7, 8) features that are important in the development of pulmonary allergic responses. Following active IgE sensitization and pulmonary allergen challenge, rats develop progressive lung inflammation,

the intensity of which significantly correlates with the development of airway obstruction as assessed by measurements of lung resistance (7). The resulting inflammatory response consists of a mixed cellular infiltrate which contains eosinophils in a perivascular and peribronchiolar distribution.

The eosinophil is felt to play an important role in many of the pathologic and physiologic abnormalities noted in asthma, including airway epithelial cell denudation and the development of airway hyperresponsiveness (9). As such, we (10) and others (11) have been interested in developing methods to analyze the mechanisms by which eosinophils contribute to these processes. Gundel et al insufflated antigen-coated Sepharose beads to induce airway eosinophilia and airway hyperresponsiveness in cynomolgus monkeys (11). We have developed a model of pulmonary eosinophilia in rats in which the animals are injected intravenously with Sephadex beads and develop intense eosinophilic infiltrates located in perivascular and peribronchial connective tissue (10).

We have now extended these initial studies to determine the effects that these histopathologic changes have on pulmonary resistance and airway responsiveness. In the design of our experiments, we utilized the methods employed by both Gundel et al. (11) and Sorden et al. (10) in order to induce tissue inflammation that was both eosinophil-rich and located either primarily within the airway or within the vascular bed, respectively. Finally, we analyzed whether either or both of these treatment regimens could affect the pulmonary response to allergen challenge in IgE-sensitized animals.

Methods

Complete details of the methods for sensitization, antigen challenge, measurements of pulmonary mechanics, methacholine challenges and histologic evaluation have been published previously (7, 8, 10, 12) and, therefore, will only be briefly summarized below. The investigators responsible for evaluating lung mechanics, airway responsiveness, and lung inflammation were not aware of the treatment and antigen group assignments of the individual animals until all data were compiled.

Animals

All methods used in these studies were evaluated and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin, and conform to the NIH "Guide for the care and use of laboratory animals". Outbred adult

male CD (Sprague-Dawley), specific-pathogen-free rats (Charles River Laboratories, Portage, MI) were used for all protocols.

Sensitization

Rats were actively sensitized to ovalbumin (OA) (Sigma, St. Louis, MO) with a single subcutaneous injection of 1 mg ovalbumin (Sigma, St. Louis, MO) and 200 mg aluminum hydroxide, along with an intraperitoneal injection of 0.5 ml killed *Bordetella pertussis* suspension (Difco, Detroit, MI), 10–14 days prior to antigen challenge. Using these methods, rats produce anti-OA IgE antibody, their sera having 48 h heat-labile passive cutaneous anaphylaxis titers of 1:4–1:64 ten to 14 days following initial sensitization (8).

Antigen challenge

Pulmonary antigen challenge was performed by insufflating OA directly into the trachea, after anesthetizing the rat with ketamine (100 mg/kg subcutaneously) (Parke-Davis, Morris Plains, NJ); bovine serum albumin (BSA) (Sigma, St. Louis, MO) was used as an irrelevant antigen. Initially, 1 mg antigen protein, dissolved in 0.1 ml saline, was insufflated; however, because 4 of the first 10 rats challenged with 1 mg OA died of severe pulmonary distress, the antigen challenge dose was changed to 0.1 mg protein for the remainder of the experiments. The physiological and histological variables were similar in the surviving rats that received 1 mg OA as in the rats that received 0.1 mg OA, and the data were thus pooled into a single OA group for analyses.

Measurements of lung mechanics

Studies of lung mechanics were conducted within the period of 18–24 h after antigen challenge; this time period was previously determined to be of value for the study of antigen-induced pulmonary inflammation in rats (7). Rats were anesthetized [ketamine 100 mg/kg + urethane 250 mg/kg (Sigma, St. Louis, MO)], instrumented with catheters in the cervical trachea and a carotid artery, paralyzed [gallamine triethiodide 30 mg/kg (Davis and Geck, Pearl River, NY) via arterial line], and ventilated mechanically to maintain a normal P_{CO_2} . Lung inflation pressure was measured at the opening of the tracheal tube, and flow and volume were determined via a constant-pressure body plethysmograph. Resistance was computed using the iso-volume method at 50% tidal volume (Buxco Model 6 Pulmonary Mechanics Analyzer, Buxco Electronics, Sharon, CT). Respiratory system re-

Table 1. Inflammation scoring criteria

Bronchioles	Alveoli	Perivascular
epithelial erosion	distribution of inflammatory cells	inflammatory cells
edema		eosinophils
inflammatory cell density	alveolar edema	
neutrophils	alveolar neutrophils	
eosinophils	alveolar eosinophils	
mononuclear cells (lymphocytes, macrophages)	alveolar mononuclear cells	

Individual scores: 0 = none, 1 = mild or focal, 2 = moderate or multifocal, 3 = severe or extensive

TSC = sum of all individual scores

ESC = sum of bronchiolar, alveolar and perivascular eosinophil scores

NSC, MSC = sums of bronchiolar and alveolar neutrophil or mononuclear cell scores

sistance (Rrs) was determined by subtracting the resistance of the tracheal tube and connector from the computed resistance.

Methacholine responsiveness

Responses to aerosolized methacholine (MeCh) were determined by ventilating the rat with 10 breaths from a second rodent ventilator in series with an ultrasonic nebulizer containing first saline, and then concentrations of methacholine in 3–10 fold increments, until the change in resistance had no further increase (maximal response). Resistance was allowed to decrease to baseline before each subsequent MeCh challenge. If a maximal response did not occur by 10 breaths of a 1.0 M MeCh concentration, additional doses of 20 or 40 breaths of 1.0 M MeCh aerosol were administered to obtain the maximal response. Methacholine responsiveness was expressed as the molar concentration necessary to provoke a change in resistance of 25% of the maximal response (PD_{25}), obtained by interpolation on a log(methacholine) vs log (Δ resistance) plot. This log-log plot was previously determined to be linear over a wider range than the conventional semi-log plot (12).

Histologic evaluation of lung tissue

After completing the methacholine challenges, the rats were killed by exsanguination, and the lungs were fixed *in situ* by perfusing the trachea at 25 cmH₂O pressure with cold Karnovsky's fixative. Sections of lung were stained with hematoxylin and eosin, and inflammatory changes were graded

in a semiquantitative manner for airways, alveoli and perivascular areas as previously described (7), except that mainstem bronchi were not included for analyses in the current studies (Table 1).

Procedures involving Sephadex G200 and Sepharose 4B

Pulmonary eosinophilia was induced with either Sephadex G200 beads (Pharmacia, Piscataway, NJ) injected intravenously 1 day before antigen challenge, or with antigen-coupled Sepharose 4B (Pharmacia, Piscataway, NJ) administered by tracheal insufflation. A sterile suspension of Sephadex G200 in saline was prepared, and 1 ml (4000 beads) was injected intravenously into ether-anesthetized rats as previously described (10). Pulmonary eosinophilia is maximal 1 day after Sephadex G200 injection (10).

Ovalbumin and BSA were coupled to Sepharose 4B as described by Gundel (11) et al. One gram of CNBr-activated Sepharose 4B was hydrated in 10^{-3} M HCl, mixed with 10 mg antigen protein dissolved in 2.5 ml coupling buffer (0.1M NaHCO₃ + 0.5M NaCl), and then rotated overnight at 4°C. The gel was washed with coupling buffer, then resuspended in coupling buffer containing 0.2M glycine. After at least 2 h, the gel was washed, consecutively, with 0.1M (pH 4.5) NaAcetate, 0.2M (pH 11.3) NaCO₃, and PBS (pH 7.4). The amount of protein coupled to Sepharose was assumed to be the difference between the starting amount and the amount present in the eluent from the first washing. Final stock suspensions containing 3 mg protein coupled to 400 mg Sepharose per ml PBS were used for antigen challenges in rats (0.1 ml insufflated into the trachea). A second batch of OA-coupled Sepharose was prepared us-

Table 2. Summary of procedures used for various treatment groups

Treatment group	Procedure A Day 0	Procedure B Day 12	Procedure C Day 13	Procedure D Day 14
BSA	OA Sensitization	i.v. saline or none	BSA Insufflation	Rrs MeCh PD_{25} Histology
OA			OA Insufflation	
i.v. Sephadex BSA challenge		i.v. Sephadex	BSA Insufflation	
i.v. Sephadex OA challenge			OA Insufflation	
Sepharose-BSA Challenge		None	BSA-Sepharose Insufflation	
Sepharose-OA Challenge			OA-Sepharose Insufflation	

Table 3. Pulmonary inflammation 18–24 h after antigen challenge.

Treatment group	N	Histology scores (mean±SD)			
		Eosinophil	Neutrophil	Mononuclear	Total
BSA	13	1.2±1.2	0.7±0.8	1.2±1.0	5.3±3.4
OA*	12	5.0±2.5	3.4±1.4	3.4±1.4	19.9±7.9
i.v. Sephadex/BSA†	10	3.6±1.0	1.5±0.5	2.0±0.8	11.4±3.2
i.v. Sephadex/OA†	10	5.3±1.3	3.0±1.5	3.5±1.2	18.8±5.9
BSA-Sepharose*	7	4.6±2.3	3.1±1.2	2.9±0.9	17.4±5.9
OA-Sepharose‡	14	5.7±2.1	4.0±0.9	4.1±0.9	22.3±4.4

* All scores increased compared with BSA group ($p<.007$)

† Eosinophil score and total score increased ($p<.03$) compared with BSA group

‡ All scores not significantly different from OA group.

ing 100 mg OA, and resulting in a final suspension containing 5 mg OA coupled to 400 mg Sepharose per ml PBS. Seven rats were challenged with each batch of Sepharose-coupled OA; the data were combined into a single group for analyses after determining that the changes in lung physiology and histology were indistinguishable between the 2 batches.

A summary of the procedures used in various treatment groups is listed in Table 2. Some of the rats in the BSA and OA treatment groups received an injection of i.v. saline under ether anesthesia to affirm that Procedure B did not introduce confounding variability. Statistical analyses were done with Systat software (version 5.0, Systat, Inc., Evanston, IL). For each rat, all inflammation sub-scores were summed to obtain a total inflammation score (TSC), and eosinophil (ESC), neutrophil (NSC) and mononuclear cell (MSC) scores were determined as the sum of the individual location scores for each cell type (7). Kruskal-Wallis analysis of variance procedures were used for comparisons among treatment groups, and preplanned pairwise comparisons (Mann-Whitney) between selected treatment groups were employed for variables in which the overall analysis of variance was significant.

Interactive stepwise regression analysis was used to determine the associations of Rrs and MeCh responsiveness with the treatments and the histologic findings. Treatment variables (antigen, Sepharose, Sephadex) were assigned qualitative values of 0 or 1 for the purpose of determining quantitative coefficient estimates, and allowing simultaneous regressions with the inflammatory cell scores (13). The variable with the highest individual correlation coefficient at each step was entered into the regression. Variables were then removed from the regression if they had an insignificant contribution to the fit. Once the significant

predictors were identified, independent variables were checked for collinearity, and residual analysis was performed to detect nonnormality or nonrandomness of errors, and any cases with excessive influence on the estimates. Two outliers were identified and deleted from the final estimate of coefficients.

Results

Pulmonary inflammation

The intensity of pulmonary inflammation, as depicted by histology scores at 18–24 h after antigen challenge, is summarized in Table 3. Compared with BSA-challenged controls, insufflation of OA or Sepharose-coupled BSA significantly increased the eosinophil, neutrophil, mononuclear cell, and total inflammation scores. In contrast, pretreatment with i.v. Sephadex beads 24 h before BSA insufflation resulted in significant increases in eosinophil and total inflammation scores, but the changes in neutrophil and mononuclear cell infiltrates were not statistically significant. Pulmonary inflammation in OA-challenged rats was not significantly enhanced by pretreatment with i.v. Sephadex or by coupling the OA to Sepharose.

The location of the inflammatory cell infiltrates differed among the various treatment groups. In Sepharose-insufflated rats, the most intensive inflammatory cells infiltrates occurred around Sepharose beads in alveoli and alveolar ducts. The infiltrates were neutrophil dominated, although eosinophils and macrophages were also present. Eosinophils were present in infiltrates dominated by lymphocytes and macrophages found in bronchiolar lamina propria and periarterial connective tissue. Similar infiltrates containing eosinophils were present around pulmonary veins.

The pulmonary inflammation that develops following Sephadex injection was reported previously from parallel experiments performed in our laboratory (10). The day after intravenous administration of Sephadex, the beads lodged almost ex-

Table 4. Resistance and methacholine responsiveness 18–24 h after antigen challenge.

Treatment group	N	Rrs (cmH ₂ O·ml ⁻¹ ·s)	MeCh PD ₂₅ (Molar)	Maximal response (cmH ₂ O·ml ⁻¹ ·s)
BSA	6	.101±.015	0.23 (1.8)	.537±.162
OA	7	.110±.028	0.10 (2.5)	.728±.253
i.v. Sephadex/BSA	6	.105±.003	0.18 (1.6)	.784±.368
i.v. Sephadex/OA	6	.126±.018	0.15 (1.3)	.621±.249
BSA-Sepharose	6	.130±.030	0.11 (3.5)	.611±.087
OA-Sepharose	13	.135±.024	0.13 (1.9)	.597±.209

Rrs and Maximal Response are mean ± SD; PD₂₅ is geometric mean (GSD).

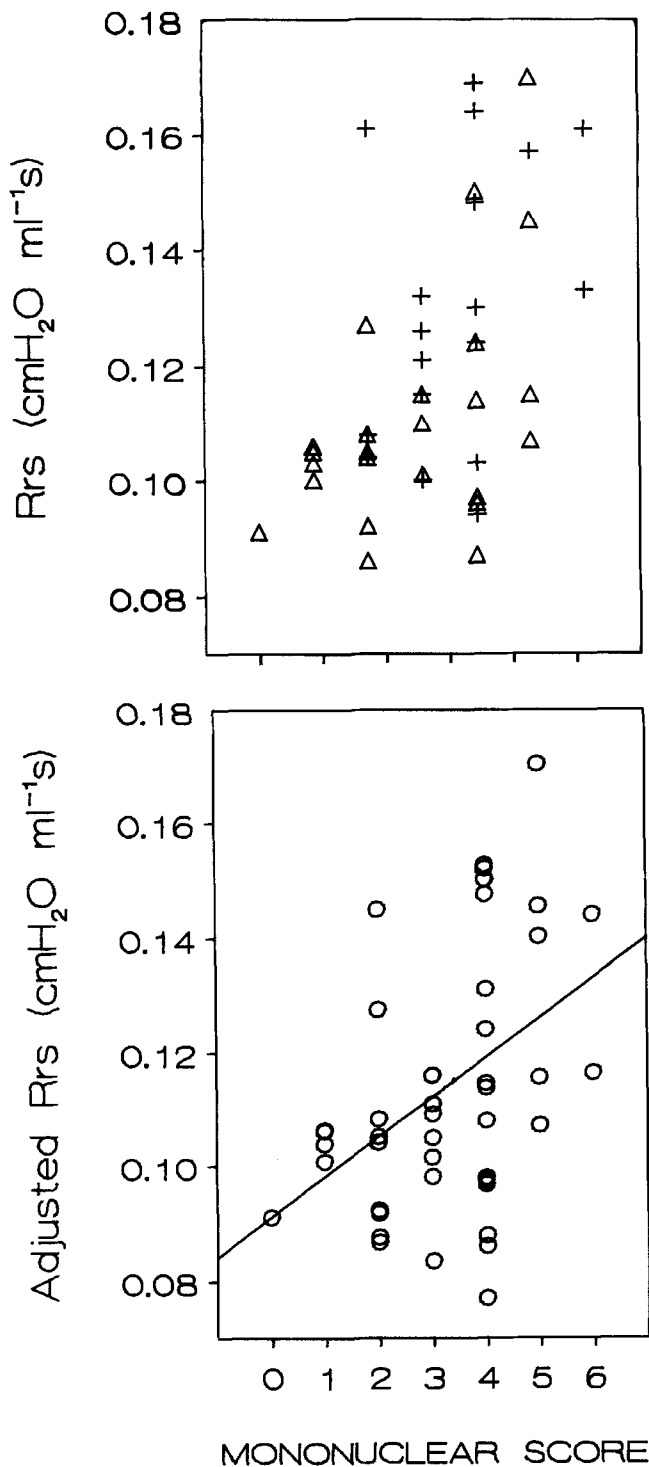


Fig. 1. Upper: Relationship of respiratory system resistance (Rrs) with mononuclear cell infiltrates in the lungs 18–24 h after challenge with Sephacoupled antigen (+) or with soluble antigen (Δ). Lower: All rats, Rrs adjusted for Sephacoupled antigen effect; regression line shows association with mononuclear cell score. Regression equation: $Rrs = 0.007(MSC) + 0.091$ ($+0.017$ for Sephacoupled), $r = 0.58$, $p = 0.0002$.

clusively in pulmonary arteries. Eosinophils were predominantly present in periarterial and in peribronchiolar locations. Lower densities of eosinophils were also present around pulmonary veins.

Resistance and methacholine responses

Pre-methacholine Rrs, the maximal responses to MeCh, and the PD_{25} for aerosolized methacholine at 18–24 h after antigen challenge are summarized in Table 4. The BSA-control group had the lowest Rrs and the highest PD_{25} ($p < .04$ for both variables, compared with all treated rats). There were no significant differences among groups for maximal response to MeCh.

Pairwise comparisons detected a significant effect on Rrs for Sephacoupled antigen ($p = .003$), but not for OA or Sephadex. Because previous studies demonstrated that Rrs correlates with the magnitude of inflammation in late allergic responses in rats (7), we utilized stepwise regression techniques to determine the predictive value of TSC, ESC, MSC, NSC, and the treatments (OA, Sephacoupled, Sephadex) on Rrs. Mononuclear cell score was found to be a significant predictor ($p = .006$) for Rrs (Fig. 1), and insufflation of Sephacoupled had a significant independent effect on Rrs ($p = .01$) above that pre-

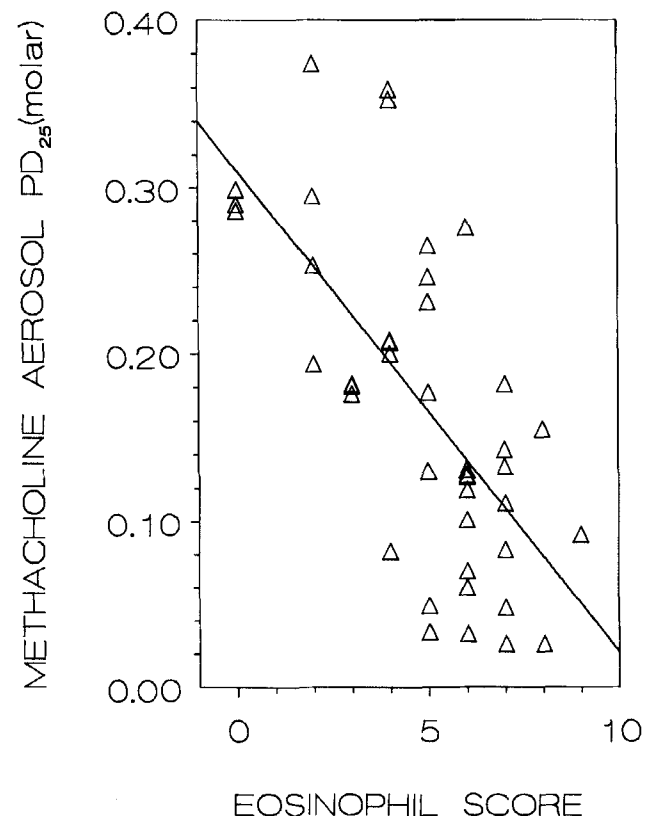


Fig. 2. Relationship of methacholine responsiveness with pulmonary eosinophil infiltrates at 18–24 h after antigen challenge. Regression equation: $PD_{25} = 0.311 - 0.029(ESC)$, $r = 0.66$, $p < 0.0001$.

dicted by mononuclear cell infiltrates. Least squares regression indicates that the range of mononuclear cell scores was associated with a 1.5 fold change in Rrs. Conversely, ESC had no significant positive correlation with Rrs, and NSC had no significant predictive value when combined with MSC, although a high degree of collinearity between MSC and NSC ($r=.75$) resulted in NSC having predictive value when all other independent variables were deleted from the equation. Challenges with OA or Sephadex had no significant independent effect on Rrs beyond that attributed to mononuclear cell infiltrates.

Stepwise regression analysis was also used to identify correlates with MeCh responsiveness. Eosinophil score was a significant predictor for PD_{25} ($p<.0001$, Fig.2). Least squares regression indicates that the range of eosinophil scores was associated with a 6.2 fold change in sensitivity to MeCh. Sephadex, Sepharose, and OA had no significant independent correlation with PD_{25} ; MSC and NSC were significant correlates only when ESC was not included in the equation, probably due to their collinearity with ESC ($r=.66$ for both MSC and NSC vs ESC).

Discussion

In previous experiments, we demonstrated that IgE sensitization and antigen challenge (7), as well as intravenous Sephadex treatment (10), can result in airway inflammatory responses which may contain variable numbers of eosinophils. In the allergen challenge studies, inflammation was evaluated at 6–10, 17–21, and 22–26 h after antigen administration. Mixed cellular infiltrates containing eosinophils were noted in the bronchus and bronchioles and similar in intensity at the time points later than 17 h. Neutrophil infiltration developed rapidly and was significantly increased at each of the late time points. Mononuclear cells and eosinophils appeared to infiltrate more gradually, being significantly increased only at the 17- to 21-h and 22- to 26-h time points.

Sephadex treatment resulted in the formation of macrophage and polymorphonuclear (predominantly eosinophils) cell aggregates around the beads trapped within the vascular bed. At 24 h post-injection, macrophages and occasional multinucleate giant cells with large numbers of surrounding eosinophils within periarterial and peribronchiolar connective tissue developed; by 48 h post-injection, eosinophil and macrophage aggregates were at their greatest density.

The purpose of this series of experiments was to expand on these observations and to determine if experimentally-induced tissue inflammation, con-

taining variable amounts and different anatomical locations (airway vs vascular bed) of eosinophils, could influence the histologic and concomitant physiologic changes that occur following pulmonary allergic responses. Antigen challenge alone, intravenous Sephadex treatment, and airway Sepharose insufflation all significantly increased eosinophil numbers within the lung. Of all the treatment regimens, intravenous Sephadex had the most selective effect on increasing tissue eosinophils. Antigen challenge in IgE-sensitized animals produced similar intense inflammatory responses, both in total inflammatory cell scores and individual inflammatory cell numbers, regardless of whether the antigen was directly insufflated into the airway in sensitized rats, in sensitized rats pretreated with Sephadex, or if the antigen was first coupled to Sepharose beads and then delivered into the airway. It is possible that differences among groups could have been observed had lower antigen concentrations of ovalbumin been used for challenge. However, in data previously published (7), at an antigen dose 10 times greater than that used in the experiments reported herein, only 10–15% of rats developed significant increases in resistance (i.e. a late response) following challenge. Thus, the dose chosen should have maximized our ability to demonstrate any additive effects of tissue eosinophilia on the histologic and physiologic response to allergen challenge.

In addition to evaluation of tissue inflammation, physiologic correlates of respiratory system resistance and methacholine responsiveness were evaluated. All treatments tended to increase Rrs compared with control animals, although the changes were small, and of the 3 treatments, only Sepharose produced statistically significant changes in a small group of animals. Thus, the experimental induction of tissue eosinophilia did not markedly augment late changes in Rrs that develop following antigen challenge alone. In terms of the relationship of any induced alterations in Rrs with inflammatory cell infiltrates, mononuclear cell numbers were significantly associated with the observed changes that occurred in all treatment groups, whereas numbers of neutrophils and eosinophils were not. Interestingly, insufflation of Sepharose beads was associated with an increase in Rrs disproportionate to that associated with mononuclear cell infiltrates.

The biologic and/or physiologic mechanisms responsible for these observed changes in Rrs cannot be conclusively determined by these initial studies. It is possible that the presence of airway inflammation, regardless of treatment, or the augmentation of this process by the anatomical location of the Sepharose beads, could have resulted in a thick-

ened airway wall or mechanical obstruction that resulted in elevations in resistance (14). There was no microscopic evidence of airways occluded by Sepharose beads, although if such occlusions existed, they may have been dislodged when lungs were filled with fixative solution. Of interest is the correlation of mononuclear cell number with resistance measurements. Lymphocytes are known to be a source of interleukin-2 which has been demonstrated to have acute effects on both lung mechanics and airway responsiveness in rats (15). Thus, the elaboration of this cytokine by airway lymphocytes may have contributed, at least in part, to the observed alterations in resistance.

In addition to abnormalities in resistance, we observed that airway responsiveness was related to the development of pulmonary inflammation. Changes in airway responsiveness to nonspecific stimuli (e.g. histamine and methacholine) have been noted to occur following allergen challenge in a number of animal models (16–19). In our experiments, only eosinophil score was a significant predictor of methacholine responsiveness indicating, at least, a temporal relationship between eosinophil ingress into tissue and alterations in airway sensitivity to cholinergic stimuli. Similar results have been observed in monkeys (11), guinea pigs (20) and rats (21). Gundel et al. (11) utilized a series of intratracheal instillation of *Ascaris suum*-coated Sepharose 4B beads administered once a week for 4 weeks in naturally sensitized cynomolgus monkeys. Using bronchoalveolar lavage and airway response to methacholine, they noted a concurrent increase in airway eosinophils with the development of airway hyperresponsiveness. Our experimental methods differed based on the fact that only one instillation of Sepharose beads was performed and inflammation was assessed using histopathology.

Following an initial ovalbumin sensitization in guinea pigs, Ishida et al. (20) used repetitive antigen challenge to induce airway hyperresponsiveness that was associated with tissue eosinophilia in guinea pigs. In contrast to our results, the eosinophil infiltration occurred primarily within the epithelium; however, no changes in epithelium by either morphological or morphometric criteria were observed, suggesting that the eosinophil was not affecting acetylcholine response due to the loss of relaxing factors or the exposure of irritant receptors within the airway.

In rats, Laycock et al. (22) used initial and booster injections of intravenous Sephadex beads that resulted in a dose-dependent increase in numbers of eosinophils located in bronchoalveolar lavage fluid and lung tissue. These changes were associated with an increased response to intravenous

5-hydroxytryptamine, in which respiratory airflow was assessed by expressing increases in overflow volume as a percent of the maximum response. In contrast to our evaluations, the effects of other inflammatory cell types on these measurements were not reported.

Although the location of eosinophils within the lung in our experiments does not precisely duplicate what has been reported in patients with severe (22) or well-controlled (23, 24) asthma, our findings do concur with others who have emphasized that the mediator repertoire of the eosinophil, and its presence and subsequent activation within asthmatic airways, may contribute to the development of airway hyperresponsiveness (9). These effects could be mediated by the actions of eosinophil granule proteins on airway epithelium (25), on bronchial smooth muscle (26) or by the generation of toxic oxygen products (27).

In conclusion, a variety of immunological and irritant challenges to the lung can result in pulmonary inflammation that may be associated with alterations in physiologic parameters such as resistance and airway response to methacholine. In the design of our experiments, we used techniques that were intended to induce tissue inflammation that was both eosinophil-rich and located either primarily within the airway or within the vascular bed. In combining the data obtained from these various techniques, we were able to determine that the presence of mononuclear cells was significantly associated with increased resistance, and that eosinophil numbers correlated significantly with airway responsiveness to aerosolized methacholine. While these relationships are complex, they provide impetus for the further definition of mechanisms by which these cells may influence these and other physiologic processes within the lung.

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