Comparisons of specific and nonspecific bronchoprovocation in subjects with asthma, rhinitis, and healthy subjects

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Background: *We studied subjects with atopic asthma, atopic rhinitis, and nonatopic healthy subjects to evaluate responsiveness to bronchoprovocation with both methacholine and allergen.*
Methods: *Subjects with a demonstrable FEV_1 PD_{20} to methacholine or allergen (responders) were further analyzed for putative sensitivity (PD_{20} FEV_1) and reactivity (dose-response slopes) to determine whether any characteristics could distinguish individuals with asthma from other responders. Subjects were recruited without sex restrictions and were between the ages of 18 and 45 years old. They were nonsmokers, had no other medical problems, and were free of upper respiratory infection for at least 6 weeks before challenge. All had a history taken, physical examination, limited laboratory screening, chest radiography, pulmonary function testing, and intradermal skin testing before admission to the study.*
Results: *Although the groups were significantly different in both sensitivity and reactivity to methacholine, responses to allergen bronchoprovocation were sufficiently similar between responders with asthma and those with rhinitis to prevent separation on the basis of either sensitivity or reactivity. The fall in FEV_1 at the nadir of the late response, which was greater in the asthma group, was significantly correlated with sensitivity and reactivity of the immediate response to allergen but not to methacholine. Regression analysis demonstrated a stronger association between allergen and methacholine responsiveness in subjects with rhinitis than in subjects with asthma.*
Conclusion: *We concluded that (1) nonspecific bronchial hyperresponsiveness fails to explain why patients with allergic asthma have clinical asthma as a result of allergen exposure and patients with allergic rhinitis do not; (2) hyperresponsiveness to allergen does not simply reflect quantitative or qualitative airway nonspecific hyperresponsiveness; and (3) clinical asthma may involve mechanisms difficult to elucidate by laboratory bronchoprovocation techniques.*
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Key words: *Bronchoprovocation, methacholine, allergen, asthma, rhinitis*

Patients with allergic asthma have hyperresponsive airways to both nonspecific agents and specific allergen after inhalational challenge. What distinguishes such patients from allergic individuals without asthma is uncertain, because many atopic individuals without clinical manifestations of asthma also have positive inhalational responses to methacholine and to appropriate allergens. A testable hypothesis is that the character of an asthmatic individual's airways hyperresponsiveness is quantitatively or qualitatively different from that of the allergic hyperresponsive individual without asthma. Asthmatic and nonasthmatic controls have been compared in their responsiveness to either nonspecific agents (methacholine or histamine) or specific allergens,[1-5] and correlations between responsiveness to methacholine and allergens have been found in patients with atopic asthma.[6,7] We are not aware, however, of previous studies comparing bronchial sensitivity and reactivity in the same asthmatic and nonasthmatic individuals with positive responses (i.e., documented PD_{20} FEV_1) to both agents.

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Abbreviations used

FEV_1: Forced expiratory volume during the first second of the forced vital capacity (FVC)
PD_{20}: Cumulative provocative dose in breath units (BU) causing a 20% fall in FEV_1
APD_{20}: Allergen-provoked PD_{20}
ASlope: Allergen-provoked slope of the least squares line fitted to all dose-response points
ATSlope: Allergen-provoked "terminal" slope of the line connecting the last two dose-response points
MPD_{20}: Methacholine-provoked PD_{20}
MSlope: Methacholine-provoked slope of the least squares line fitted to all dose-response points
MTSlope: Methacholine-provoked "terminal" slope of the line connecting the last two dose-response points
r_s: Spearman's rank correlation coefficient: the product-moment correlation of data ranks
R^2: Coefficient of determination: the square of product-moment (Pearson) correlation coefficient

Analysis of dose-response changes during bronchoprovocation and comparisons with in vitro muscle preparations have led to notions of sensitivity and reactivity[1]: that is, the dose required to achieve an arbitrary change, and the vigor or strength of the response as measured by the slope of the dose-response curve, respectively. We postulated that PD_{20} and slope, although correlated, assess different mechanisms, and that the analysis of both would be useful in evaluating airway responses. The asthmatic response was expected to be both more sensitive and more reactive, and responsiveness to methacholine would predict responsiveness in subjects with asthma but not in those with rhinitis.[7]

Previous reports indicate that groups of patients with asthma and patients with rhinitis can be distinguished by nonspecific challenge,[2, 3] but that they are indistinguishable in sensitivity to allergen bronchoprovocation.[4, 5] Whether asthmatic patients respond by a more vigorous, hyperreactive response than do other individuals responsive to nonspecific or specific challenges has not been systematically addressed. We initially studied three groups of subjects: Subjects with atopic asthma, subjects with atopic rhinitis, and normal (healthy), nonatopic asymptomatic controls, by use of both nonspecific and specific bronchovocation to compare responsiveness of patients with allergic (atopic) asthma and nonasthmatic patients. Subjects with asthma and those with rhinitis responsive to methacholine and allergen were analyzed for between- and within-group comparisons, and were further compared for the predictive value of methacholine responsiveness and skin reactivity in determining bronchial responsiveness to allergen. We also evaluated measures for association with late responses to allergen bronchoprovocation.

TABLE I. Responsiveness of subjects challenged

	Subject groups		
Challenges	**Asthma**	**Rhinitis**	**Normal**
Methacholine	25/25 (100%)*	35/75 (47%)	16/66 (24%)
Allergen	24/25 (96%)	18/29 (62%)	0/23 (0%)

$n = 166$.
*Numbers indicate no. responders/no. challenged (= % responders). Responders defined as subjects demonstrating at least a 20% drop in FEV_1 from diluent baseline during standard challenge with methacholine or allergen.

TABLE II. Allergens used in bronchoprovocation*

	Asthma		Rhinitis		Normal	
Allergens	+	−	+	−	+	−
Ragweed	10	1	16	2	0	7
Alternaria	9	1	0	5	0	8
Mite	3	0	1	3	0	6
Timothy	0	0	1	0	0	0
Horm	0	0	0	1	0	0
Dog	1	0	0	0	0	1
Cat	1	0	0	0	0	1
Totals	24	2	18	11	0	23

*Asthmatic subjects and subjects with rhinitis had positive wheal and flare skin reactions to allergens used in bronchoprovocation; one asthma subject challenged with two different allergens. Normal subjects had negative skin reactivity.

METHODS

Subjects

We studied 166 volunteer subjects composed of 25 individuals with asthma, 75 with atopic rhinitis, and 66 normal, nonatopic asymptomatic controls. Protocols were approved by the University of Iowa Institutional Review Board for human studies, and informed consent was obtained from all subjects. Each subject was challenged with methacholine, and 77 were subsequently challenged with allergen. Subjects were recruited without sex restrictions and were between the ages of 18 and 45 years. They were nonsmokers, had no other medical problems, and were free of upper respiratory infection for at least 6 weeks before challenge.

TABLE III. Between-group comparisons of dose-response slopes in all subjects

Group	Parameters compared			
	MSlope	MTSlope	ASlope	ATSlope
Asthma	−9.28 ± 1.62*	−7.73 ± 1.45	−3.79 ± 0.91	−3.90 ± 0.90
	n = 25	n = 25	n = 25	n = 25
Rhinitis	−0.38 ± 0.09	−0.32 ± 0.07	−0.87 ± 0.29	−1.01 ± 0.38
	n = 75	n = 75	n = 29	n = 29
Normal	−0.14 ± 0.04	−0.10 ± 0.03	—	—
	n = 66	n = 66		
Statistical analysis				
Asthma vs Rhinitis	p = 0.0001†	0.0001	0.009	0.01
Asthma vs Normal	p = 0.0001	0.0001	—	—
Rhinitis vs Normal	p = 0.0081	0.0083	—	—

n = 166.

M, Methacholine; *A*, allergen; *Slope*, least squares line fitted to all points (minimum no. of points = 3); *T slope*, line connecting last two points.

*Numbers are mean ± SEM; n = numbers of subjects in each group.

†p is probability with nonparametric Wilcoxon two-sample test; identical p values were obtained when data were log transformed.

All had a history taken, physical examination, limited laboratory screening, chest radiography, pulmonary function testing, and intradermal skin testing before admission to the study.

Subjects with atopic asthma satisfied criteria for the diagnosis of asthma suggested by the American Thoracic Society.[8] They had positive intradermal skin tests to one or more relevant allergens (ragweed, timothy, *Alternaria, Dermatophygoides pteronyssinus*, and *D. farinae*, dog, cat) and FEV_1 60% or greater predicted before challenge. Fluctuations of FEV_1 20% or greater had been documented spontaneously or after inhaled bronchodilator. They had symptoms of mild extrinsic asthma and were clinically stable at the time of bronchial challenge. At the time patients were studied they were not on inhaled or systemic steroids and were not dependent on steroids. Medications such as theophylline were stopped 48 or more hours before each challenge. β_2-agonist inhalers were stopped 12 or more hours before each challenge. None were on antihistamines.

Subjects with atopic rhinitis were defined as having positive intradermal skin tests to relevant antigens (ragweed, mite, and timothy) and history and findings consistent with allergic rhinitis but no symptoms or history of asthma. Ragweed constituted the relevant allergen in 16 of the 18 subjects with rhinitis, and, to eliminate unrecognized asthma, subjects sensitive to ragweed had been followed weekly throughout the ragweed season without symptoms of asthma or changes in spirometry. The studies presented in this report were done out of season at which time the subjects were asymptomatic and on no medication.

Nonatopic aymptomatic subjects had negative skin tests to a battery of 16 common inhalants, no history, symptoms, or findings of asthma or rhinitis, and none were taking medications.

Skin tests

Skin testing was performed by intradermal injection of approximately 0.02 ml of allergen extract in the upper outer arm. After 20 minutes, a 5 × 5 mm wheal greater than the diluent was considered a positive test.

To determine atopic status, we used a battery of 16 common inhalants plus diluent (0.9% NaCl and 0.4% phenol) and positive controls (codeine phosphate 25 mg/ml and histamine phosphate 0.1 mg/ml). Extracts were purchased from Greer Laboratories (Lenoir, N.C.), Center Laboratory (Port Washington, N.Y.), and Berkeley Biologicals (Berkeley, Calif.). Screening concentrations were 1000 protein nitrogen units (PNU)/ml for molds, 100 PNU/ml for pollens and danders, and 100 allergy units per milliliter for house dust mites; wheal diameter was recorded to these initial concentrations, and this measure was used in the analysis of skin reactivity. When screening skin tests were positive, titrations were determined in most of the subjects; end point was defined as PNU concentration per milliliter resulting in a wheal less than 5 mm above diluent.

Inhalational challenges

All subjects gave informed consent before challenges, and the method of Chai et al.[9] was used. All challenges were done out of season. Allergen challenges took place between 7 and 10 AM to avoid diurnal variations in results. Methacholine challenges were performed throughout the work day, and each subject had at least three highly reproducible tests, medians of which were used for analysis.

Both allergen and methacholine challenges were performed with use of a DeVilbiss 646 nebulizer powered by 20 psi of compressed air (DeVilbiss Co., Somerset, Pa.) and a Rosenthal-French dosimeter (Johns Hopkins University, Baltimore, Md.). The nebulization time per puff was

TABLE IV. Comparison of responses to methacholine and allergen in all responder subjects*

Group	MPD_{20}	MSlope	MTSlope	APD_{20}	ASlope	ATSlope
Asthma	4.7 ± 1.4†	−9.28 ± 1.6	−7.73 ± 1.4	133 ± 46	−4.26 ± 0.98	−4.39 ± 0.96
	n = 25	n = 25	n = 25	n = 24	n = 24	n = 24
Rhinitis	62 ± 9	−0.76 ± 0.18	−0.68 ± 0.12	60 ± 23	−1.39 ± 0.42	−1.62 ± 0.57
	n = 35	n = 35	n = 35	n = 18	n = 18	n = 18
Normal	82 ± 14	−0.42 ± 0.12	−0.37 ± 0.11	—	—	—
	n = 16	n = 16	n = 16			
Asthma vs Rhinitis	p = 0.0001	0.0001	0.0001	NS	NS	NS
Rhinitis vs Normal	p = NS	NS	NS	—	—	—
Asthma vs Normal	p = 0.0001	0.0001	0.001	—	—	—

*No. of subjects *(n)* reflect responders qualifying for analysis in each group as shown in Table I.
†No., abbreviations and statistical analysis are as noted in Table III.
NS, Not significant.

0.6 seconds. The volume of solution in the nebulizer was 1 ml of each dilution. The output of the nebulizer was 0.025 ± 0.002 ml per puff.

The best of three efforts for baseline FEV_1 and FVC after inhalation of diluent was obtained for each subject on a Jones Pulmonaire (Oak Brook, Ill.), and the percent predicted was calculated from the chart of Bates and Christie.[10] Spirometric values were similarly obtained 3 to 5 minutes after inhalation for methacholine and at 12 to 15 minutes after inhalation for allergen. Mini-Wright (Clement Clarke International, Harlow, U.K.) peak flow recordings were also obtained concurrently to establish baseline values for later monitoring when used out of hospital. Inhalation was from functional residual capacity to inspiratory capacity. Individuals inhaled each dose of methacholine over 5 seconds and then exhaled to functional residual capacity without breathhold. Five consecutive breaths for each concentration were performed in this fashion. Subjects were in a seated position, and nose clips were not used. Methacholine concentrations were 0.075, 0.15, 0.62, 2.5, 5.0, 10.0, 25.0 mg/ml with use of diluent containing 0.9% NaCl, 0.4% phenol. Doses were administered at 5-minute intervals until a drop in FEV_1 20% or greater was obtained as compared with diluent response, or until 25 mg/ml was achieved. Initial allergen concentrations for inhalational challenge were based on the results of intradermal skin testing. Allergens were chosen that best matched the subject's seasonal history and skin test results; the initial concentration chosen was one tenth of that producing a 5 × 5 mm wheal above diluent on intradermal skin testing. Normal subjects were randomly challenged with representative extracts for control purposes. Most of the challenges involved *Alternaria* and ragweed (Greer Laboratories) and *D. farinae* (Berkeley Biologicals). Allergen concentrations during bronchial challenges were increased 10-fold until a 10% drop in FEV_1 occurred, followed by two-fold increments administered until a 20% decrease in FEV_1 was noted, or until a concentration of 5000 AU/ml for *D. farinae* or 10,000 PNU/ml for the other allergens was reached. After completion of allergen challenge, subjects were monitored with spirometry, physical assessment, and direct observation by the nursing staff in the Clinical Research Center. As a safety precaution, asthmatic patients were observed for at least 24 hours after allergen challenge, with FEV_1, FVC, and peak flow recordings (Mini-Wright peak flow meter) every 15 minutes until recovery of FEV_1 to within 10% range of the diluent response, followed by hourly spirometry and peak flow recordings for 8 hours, every 2 hours for 8 more hours, and every 4 hours until discharge from the hospital or more often if symptoms or spirometric abnormalities occurred.

A *late response* was defined as a drop in FEV_1 of 15% or more after initial recovery from the early response, but no sooner than 2 hours after allergen challenge. The nadir of the late drop in percent FEV_1 from baseline was recorded and used in analysis.

$Beta_2$-agonist inhalers were used, after methacholine challenge only, if subjects became uncomfortably symptomatic or the FEV_1 remained below 80% of baseline. Subjects who were challenged with allergen did not receive $beta_2$-agonists immediately after challenge so that monitoring for late-phase responses could be done. Once a late-phase response was obtained, $beta_2$-agonists were used intermittently for symptomatic control. Patients with rhinitis and nonatopic patients were monitored for 10 hours after challenge with the same routine described for asthmatic patients. After discharge from the hospital Mini-Wright peak flow meter recordings were done on an hourly basis by the subject until bedtime. On awakening, an additional two Mini-Wright peak flow meter recordings were obtained within 1 hour. At least 4 weeks elapsed after allergen challenge was performed before further inhalational challenge tests were done.

TABLE VA. Data summary on subjects hyperresponsive to both methacholine and allergen (asthma group)

Subject	Sex	Age	%Pred FEV_1	$FEV_1/FVC\%$	MPD_{20}	MTSlope
1	M	23	83	77	0.63	−34.67
2	M	29	88	84	2.27	−8.71
3	F	20	97	88	0.38	−53.3
4	F	35	76	64	1.19	−7.4
5	F	20	90	90	0.67	−24
6	F	24	94	70	2.68	−6.1
7	F	22	110	92	2.09	−6.77
8	F	28	97	72	5.27	−2.26
9	F	21	77	69	1.46	−18.2
10	F	24	86	86	4.04	−5.16
11	M	23	68	67	0.79	−20
12	F	27	110	82	3.26	−5.16
13	F	25	96	81	3.76	−3.2
14	M	32	70	70	3.3	−3.23
15	F	32	96	86	3.99	−2.9
16	M	22	82	90	2.27	−5.97
17	F	19	107	86	1.96	−5.65
18	F	32	103	86	23.9	−1.96
19	F	24	103	92	4.88	−1.52
20	F	19	81	78	29.2	−0.4
21	F	20	103	92	4.88	−1.52
22	F	19	107	86	1.96	−5.65
$\bar{x}$ ± SEM		25 ± 1	92 ± 3	81 ± 2	4.9 ± 1.5	−7.4 ± 1.5

All subjects listed had at least three points for calculation of slopes.
Data analysis used Wilcoxon two-sample test comparing asthma and rhinitis groups; p values were identical when data were log transformed.
Groups tabulated by decreasing steepness of allergen terminal slopes (ATSlope); abbreviations as noted in Table II.

TABLE VB. Data summary on subjects hyperresponsive to both methacholine and allergen (rhinitis group)

Subject	Sex	Age	%Pred FEV_1	$FEV_1/FVC\%$	MPD_{20}	MTSlope
1	F	30	104	91	12.6	−1.12
2	M	44	91	89	12	−1.12
3	F	23	103	85	61.1	−0.35
4	M	37	107	87	24.5	−0.8
5	M	37	100	91	11.6	−2.24
6	M	30	98	67	87.2	−0.22
7	M	27	98	91	72.4	−0.29
8	F	35	100	83	9.4	−2.3
9	M	23	89	82	40.8	−0.87
10	M	30	98	67	87.2	−0.22
11	M	37	109	81	91.1	−0.24
12	M	21	111	89	18.7	−0.96
13	F	24	93	79	178	−0.05
$\bar{x}$ ± SEM		31 ± 2	100 ± 2	83 ± 2	54 ± 14	−0.83 ± 0.21
Wilcoxon	p =	0.0107	0.0555	0.6440	0.0001	0.0001

Responders to methacholine and allergen were defined as those individuals who achieved a 20% or greater drop in their FEV_1 from diluent FEV_1. PD_{20} was defined as cumulative breath units (CBUs) resulting in a 20% or greater decline in FEV_1. One breath of 1 mg/ml of methacholine constituted 1 BU, and one breath of 100 PNU/ml of allergen constituted 1 BU. By definition, all "responsive" subjects achieved a PD_{20} FEV_1, that is, had a fall of at least 20%

Allergen	APD_{20}	ATSlope	Late resp %drop FEV_1	Skin RXN wheal (mm)
RAG	1.23	−15	21	12
RAG	1.38	−13.2	45	20
ALT	2.47	−12	35	10
MITE	1.4	−11	39	13.5
RAG	2.53	−7.6	50	25
ALT	4.62	−7.3	11	10
RAG	1.72	−7.2	26	10
MITE	3.86	−7.05	32	8
ALT	5.24	−5.7	39	18.5
ALT	3.98	−5.4	35	25
ALT	5.98	−4.4	50	10
MITE	9.59	−2.35	16	6
ALT	24.6	−2	36	10
RAG	23.5	−1.69	0	13.5
ALT	36.3	−0.52	0	10
RAG	84.1	−0.38	25	5
ALT	295	−0.12	26	10
RAG	294	−0.06	23	7
RAG	244	−0.04	8	8
RAG	778	−0.04	0	11
CAT	668	−0.03	5	5
RAG	547	−0.01	7	4
	138 ± 50	−4.7 ± 1	24 ± 3	11 ± 1.2

Allergen	APD_{20}	ATSlope	Late resp %drop FEV_1	Skin RXN wheal (mm)
RAG	3.4	−10.23	24	15
RAG	7.13	−3.4	10	19
RAG	14.6	−1.96	34	13.5
RAG	12	−1.08	10	
RAG	17.4	−0.95	6	10
MITE	15.3	−0.92	21	15
RAG	24.7	−0.78	1	20
RAG	13.1	−0.6	12	22.5
RAG	71.6	−0.6	10	
RAG	51.3	−0.43	4	15
RAG	134	−0.38	8	
RAG	66.1	−0.36	6	
RAG	118	−0.18	1	15
	42 ± 12	−1.7 ± 0.8	11 ± 3	16 ± 1
	0.4029	0.2822	0.0327	0.0101

from diluent FEV_1. Slopes for methacholine were calculated as the percent decline in FEV_1 per CBU. Slopes for allergen were calculated as the percent decline in FEV_1 per 10 CBUs. Computer analysis calculated and evaluated two slopes (baseline used was after diluent FEV_1): (1) Slope: the slope of the least squares line fitted to all the points; and (2) Terminal or TSlope: the slope of the line connecting the last two points, which straddled the PD_{20}, and postulated as

TABLE VI. Spearman's rank correlation coefficients for asthma and rhinitis subjects

Parameter	APD_{20}	MPD_{20}	ATSlope	MTSlope	Late drop FEV_1 (%)	Wheal
APD_{20}	X	0.48	0.97	0.67	−0.60	−0.57
MPD_{20}	0.65	X	0.50	0.93	−0.41	−0.17
ATSlope	0.87	0.50	X	0.70	−0.61	−0.60
MTSlope	0.54	0.98	0.45	X	−0.53	−0.32
Late drop FEV_1 (%)	−0.63	−0.35	−0.65	−0.35	X	0.45
Wheal	−0.18	−0.16	0.24	−0.14	−0.25	X

r_s Shown are those for asthmatic subjects (above Xs) and subjects with rhinitis (below Xs) hyperresponders listed in Table V. Numbers denote r_s for all subjects (asthma $n = 22$; rhinitis $n = 13$; except rhinitis $n = 9$ for wheal). Values for r_s identical whether linear or log-transformed formed data used.

X, Denotes irrational comparison between the same two groups (e.g., APD_2. vs APD_{20}).

being more likely to reflect reactivity. All patients included in this report had at least three points for each challenge to derive slopes.

Data analysis

Four primary measures were used: (1) PD_{20} for allergen (APD_{20}) and methacholine (MPD_{20}); (2) slopes for allergen and methacholine (ASlope and MSlope); (3) Terminal (T) slopes for allergen and methacholine (ATSlope and MTSlope); and (4) wheal diameters of the screening allergen skin tests. For each inhalational challenge, a least squares line was fitted to the dose and percent FEV_1 data; log-transforming the doses produced reductions in the average R^2 (>0.85) for responders and was not used at this stage for purposes of this report.[7] Comparisons were made, however, with log-transformed dose that resulted in identical statistical conclusions. Statistical analysis of these four indicators consisted of the following: (1) unpaired comparisons of the subject groups; (2) paired comparisons of each subject's methacholine and allergen responses; and (3) a regression analysis imitating and extending that of Cockcroft et al.[7] We also analyzed the following measures for between-group and within-group comparisons: baseline percent predicted FEV_1, baseline FEV_1/FVC percent, and percent drop in FEV_1 from diluent baseline at the nadir of the late response. Nonparametric tests were carried out for the between- and within-group nonregression analyses because the normality and equal variance assumptions of the parametric procedures were questionable in a few categories. Skewness of the data toward lower values justified the use of nonparametric tests and was the reason of log-scale plots, which are shown in the figures. For comparison of two groups in independent samples, the Wilcoxon rank sum test was used. Spearman's rank correlation (r_s) was used to determine the degree of relationship between two dependent variables in paired samples.[11] For these nonparametric ranking tests the results are identical whether linear or log-transformed data are used. In most analyses the same results were obtained with parametric procedures. In general, r_s values of 0 to 0.25 indicate little or no relationship, 0.25 to 0.50 a fair relationship, from 0.50 to 0.75 a good relationship, and above 0.75 an excellent relationship, depending on the number of observed pairs.[11] All analyses were carried out with use of procedures from the SAS software package.[12] Figures with untransformed data were plotted on a log scale for illustrative purposes only.

RESULTS

Of the 166 subjects studied initially with methacholine bronchoprovocation, 76 (46%) had at least a 20% drop in FEV_1 from diluent baseline (methacholine responders). Forty-two of 77 subjects subsequently challenged with inhaled allergens had positive early responses, also defined by at least a 20% drop in FEV_1 (allergen responders). Challenge results in each subject group are summarized in Table I, and allergens used in bronchoprovocation are listed in Table II.

Comparisons of total group responsiveness

Dose-response slopes in all challenged subjects. Analysis of the data obtained in all of the 166 subjects in the three groups required use of dose-response slopes, because by definition PD_{20} could be derived only for responders. Between-group comparisons of slopes are shown in Table III. For all subjects who were challenged, significant differences between the three groups were found for methacholine slopes (MSlopes and MTSlopes). Asthma and rhinitis groups differed also in dose-response slopes generated by allergen challenge. Normal subjects had minuscule slopes to allergen that were not included for statistical analysis. Very little difference was found between Slope and TSlope in any group, but there was a tendency for methacholine TSlopes to be shallower than MSlopes in all three groups; allergen slopes (ASlope and ATSlope) tended to steepen very slightly in both the asthma and rhinitis groups.

Between-group comparisons in all responder sub-

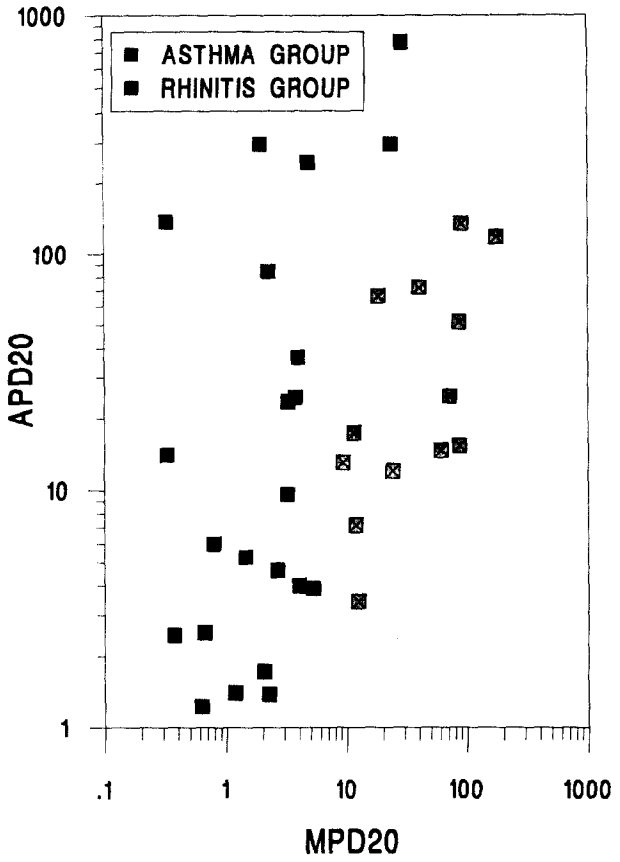

FIG. 1. Relationships between MPD_{20} and APD_{20} for individual responders to both methacholine and allergen listed in Table V. r_s showed significant relationships between MPD_{20} and APD_{20} for both rhinitis ($r_s = 0.65$, $p = 0.017$) and asthma ($r_s = 0.48$, $p = 0.023$) groups. Figure emphasizes separation of the two groups with little overlap in MPD_{20} values, with rhinitis patients being less sensitive (higher MPD_{20}), whereas the two groups overlap completely in APD_{20} values. Untransformed values are plotted on a log scale for illustrative purposes only; r_s and p were the same for linear and log-transformed data.

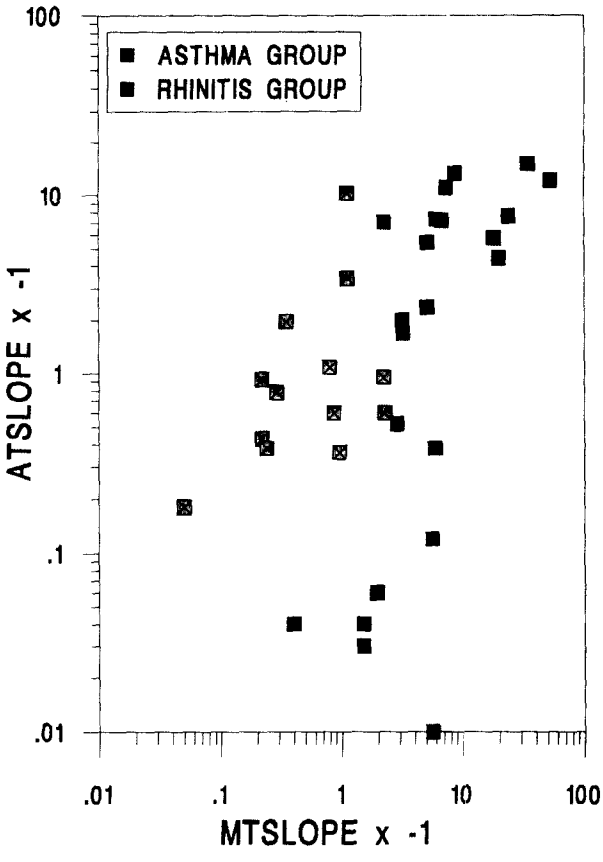

FIG. 2. Relationships between MTSlope and ATSlope for the individual responders listed in Table V. r_s statistically significant for asthmatic subjects ($r_s = 0.70, p = 0.0003$) but not for subjects with rhinitis ($r_s = 0.45$, $p = 0.13$). Figure shows separation of the two groups with minimal overlap and steeper MTSlopes for asthmatic patients but complete overlap of the values for ATSlopes. Untransformed values multiplied by -1 to facilitate log plots used simply for illustrative purposes; r_s and p were the same for linear and log-transformed data.

jects. We limited further comparisons between groups to responder subjects only, because of our interest in evaluating whether asthmatic responsiveness differs in any discernible way from nonasthmatic responsiveness. Because the groups were not balanced between men and women, we compared characteristics of airways responsiveness with methacholine and allergens in responder men and women in the asthma, rhinitis, and normal groups. No significant differences were found in this analysis, nor in group comparisons of baseline predicted FEV_1 or FEV_1/FVC, giving assurance that any differences that occurred were on some other basis.

Table IV summarizes between-group comparisons of responsiveness in all subjects responsive to either methacholine and allergen bronchoprovocation. Results showed highly significant differences between groups with asthma and groups with rhinitis for MPD_{20}, MSlope and MTSlope, but not for APD_{20}, ASlope, or ATSlope, although the rhinitis group tended to be more sensitive than the asthmatic group to allergen bronchoprovocation (lower APD_{20}) but less reactive (shallower slopes); neither of these comparisons were statistically significant. No difference in number of points for allergen slopes was found between patients with asthma and patients with rhinitis, (mean ± SEM: 6.0 ± 0.3 vs 6.8 ± 0.4, $p = 0.16$).

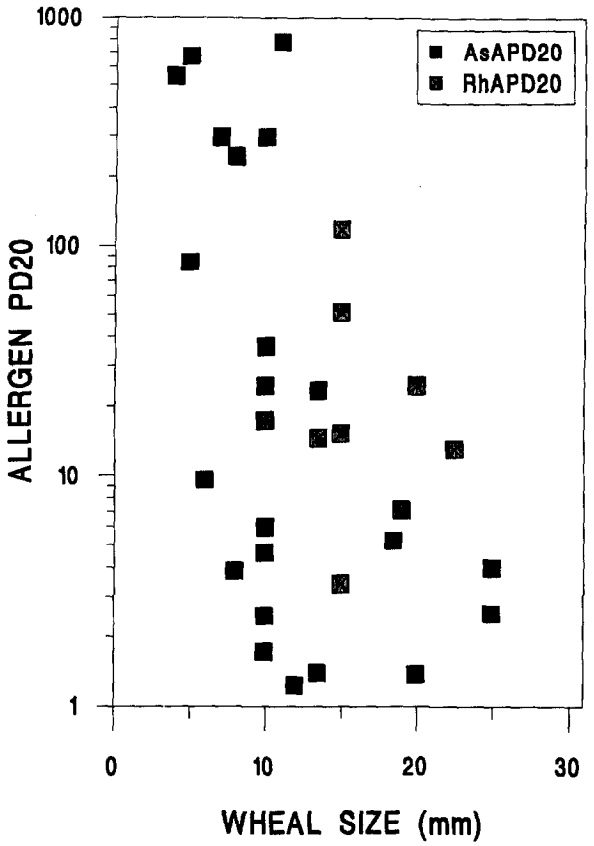

FIG. 3. Relationships between skin test wheal size and APD_{20} in asthma and rhinitis groups listed in Table V. r_s showed a significant relationship in the asthma group ($r_s = 0.57$, $p = 0.005$) but not the rhinitis group ($r_s = -0.18$, $p = 0.64$). When all subjects were included for analysis, a significant relationship was found ($r_s = -0.43$, $p = 0.015$). Untransformed values for allergen PD_{20} are plotted on a log scale for illustrative purposes only; r_s and p values are the same for linear and log-transformed data.

Between-group comparisons and within-group (paired) correlation analyses of subjects responsive to both methacholine and allergen

Data on individual asthma and rhinitis subjects responsive to both methacholine and allergen are given in Table V. These subjects or subsets thereof were used for the remainder of the analyses. Between-group comparisons were analyzed and revealed significant group differences for MPD_{20}, MTSlope, percent drop FEV_1 in late response, and skin reactivity as given in Table V. These results were similar to those for the total groups and for groups limited to ragweed challenges.

Correlation analysis was carried out to evaluate whether intragroup relationships existed for measured parameters; findings are summarized in Table VI and illustrated for selected measures in Figs. 1 to 4. Both subjects with asthma and those with rhinitis showed excellent linear relationships between PD_{20} and TSlope for both allergen and methacholine. A significant relationship was also found between APD_{20} and MPD_{20} in both groups with asthma and rhinitis, which was better for subjects with rhinitis (Fig. 1). The subjects with asthma, on the other hand, showed a better relationship than did subjects with rhinitis between MTSlope and ATSlope (Fig. 2), and between wheal size and APD_{20} (Fig. 3). Data were complete for wheal size in all subjects with asthma and nine subjects with rhinitis, and skin test titers were considered complete and reliable in 19 asthmatic subjects and 11 subjects with rhinitis. Correlations between wheal size and titer were good ($r_2 = -0.70$) in asthmatic subjects ($n = 19$) and fair ($r_s = -0.49$) in subjects with rhinitis ($n = 7$). Because Spearman's nonparametric rank correlation analysis was used, log transformed and untransformed data gave identical p values.

Associations of the severity of the late response to allergen bronchoprovocation with other measures are also shown in Table VI. No significant relationship within either the asthma or rhinitis group was found between the magnitude of the late response and MPD_{20}, although combined numbers reached significance (Fig. 4, *A*), suggesting only a fair relationship between nonspecific responsiveness on bronchoprovocation and development of a late response. On the other hand, excellent correlations were found between the magnitude of the late drop in FEV_1 and APD_{20} (Fig. 4, *B*), as well as ATSlope (Table VI). Thus the more sensitive (low PD_{20}) and reactive (steep slope) the immediate response, the more likely the subject is to have a stronger late response compared with others within the group, despite the former having received a lesser allergen dose. We also found a significant correlation in asthmatic subjects but not in subjects with rhinitis between the magnitude of the late response and the magnitude of the early response to allergen indicated by the lowest FEV_1 recorded at the completion of challenge (not shown): asthmatic patients had an early percent drop in FEV_1 of 30% ± 1.4% ($\bar{x}$ ± SEM) and a late drop of 25% ± 3%, with $r_s = 0.44$, $p = 0.038$; patients with rhinitis had an early percent drop in FEV_1 of 34% ± 3% and a late drop of 11.2% ± 3% with $r_s = 0.25$, $p = 0.40$.

Regression analysis and prediction of APD_{20} from independent variables

Following the regression procedures of other investigators,[6, 7, 13] second-level least squares analyses on groups of subjects, not individuals, were performed

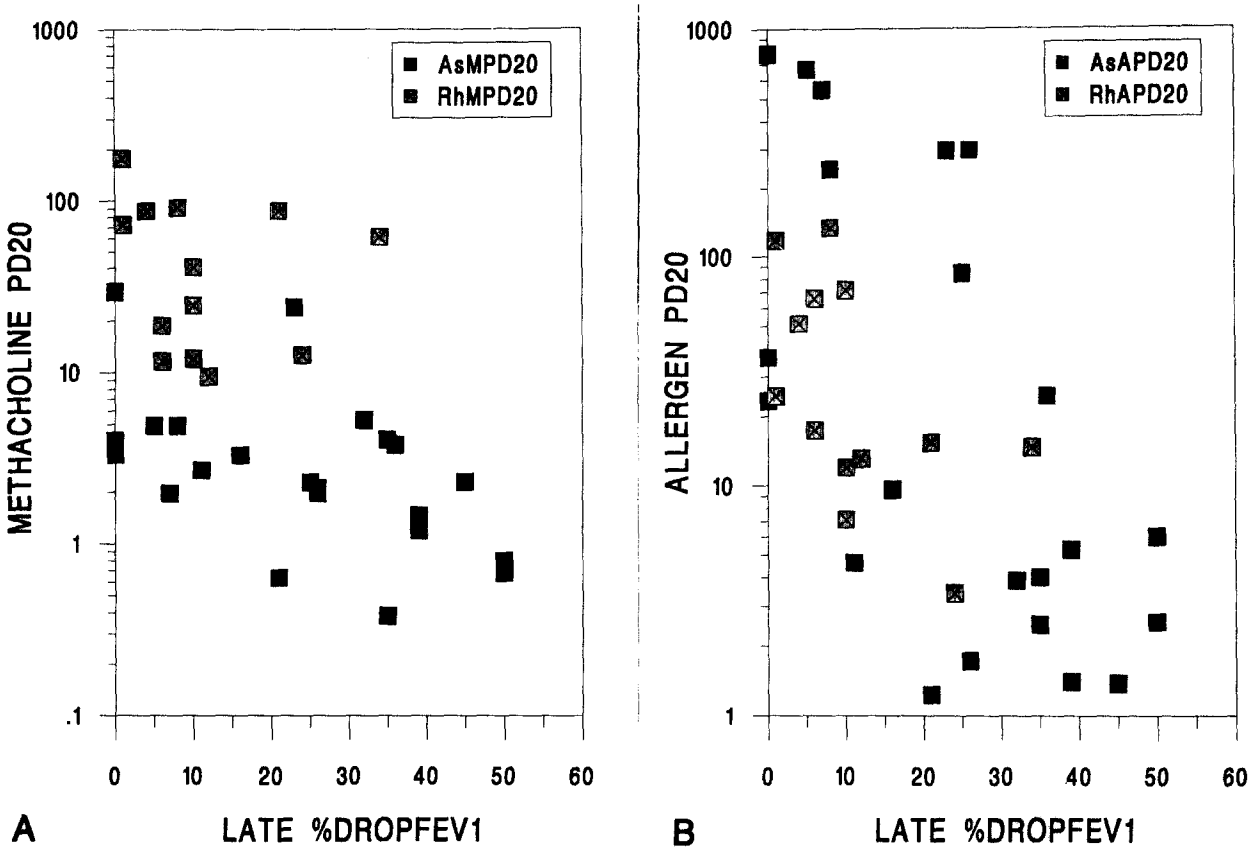

FIG. 4. Relationships between the magnitude of the late response quantitated by the percent drop in FEV_1 at its nadir and PD_{20} values for methacholine and allergen in the asthmatic subjects and subjects with rhinitis listed in Table V. If a positive late response is defined as a 15% or greater drop from baseline FEV_1, it can be seen from Fig. that 3 of 13 subjects with rhinitis (23%) and 15 of 22 subjects with asthma (68%) demonstrated a late response. **A.** Late response versus MPD_{20}. r_s failed to show significant relationships between the magnitude of late response and MPD_{20} in either asthma ($r_s = -0.41$, $p = 0.06$) or rhinitis ($r_s = -0.35$, $p = 0.24$) groups; when groups were combined; however, the relationship was significant ($r_s = -0.53$, $p = 0.0001$). Untransformed MPD_{20} values are plotted on a log scale for illustrative purposes only; r_s and p were the same for linear and log-transformed data. **B.** Late response versus APD_{20}. r_s showed significant relationships between the magnitude of the late response and APD_{20} for both asthma ($r_s = -0.60$, $p = 0.003$) and rhinitis ($r_s = -0.63$, $p = 0.02$) groups, and for all subjects combined ($r_s = -0.64$, $p = 0.0001$). Untransformed APD_{20} values are plotted on a log scale for illustrative purposes only; r_s and p were the same for linear and log-transformed data.

to identify some of the relationships among measures for allergic and nonspecific responses. Allergen PD_{20} (APD_{20}) served as the dependent variable, and skin test wheal diameter, methacholine PD_{20} (MPD_{20}), and methacholine terminal slope (MTSlope) were used as predictor variables. An empirical model-building approach was followed with use of the subjects who presented all of the regression measures. Three subjects responded to two different allergens, but each subject was allowed in a particular model analysis only once. Models were fit to all these subjects together and as separate asthma and rhinitis subgroups. In addition, within each grouping, the original measurement scales and the logarithmic transforms were used, and separate analyses were performed for either all allergens used or for ragweed as the only inhaled allergen. All possible combinations of predictor variables were examined, with R^2 (coefficient of determination) as the only measure of fit. Finally, specific multiple regression models, either determined by the best fit or those used by previous investigators,[7] were fit to various data sets and least squares regression coefficients were obtained. No tests of hypothesis were performed, so that no assumptions of normality were needed for these correlations.

Data were used for 28 subjects consisting of 20 asthmatic patients and eight with rhinitis who had all regression measures without regard to the inhaled al-

TABLE VIIA. Regression analysis: predictor of APD_{20} and $LAPD_{20}$ (untransformed variables)

	Predictors	Coefficients of determination (R^2) for APD_{20} Asthma $n = 20$	Rhinitis $n = 8$	All subjects $n = 28$
Single	MPD_{20}	0.54	0.89	0.007
	MTSlope	0.13	0.29	0.05
	Wheal	0.11	0.02	0.12
Multiple	MPD_{20} + MTSlope	0.54	0.97	0.05
	MPD_{20} + Wheal	0.59	0.89	0.14
	MPD_{20} + MTSlope + Wheal	0.59	0.97	0.19

lergen. Essentially the same pattern of results was obtained when data were limited to ragweed only (data not shown). Table VII presents single regression models and selected multiple models; the coefficient of determination is labeled R^2 for both the simple (the square of the Pearson correlation coefficient for the single predictors) and the multiple models, and represents the relative contribution the predictor(s) make to determination of the dependent variable APD_{20}. The best multiple models are presented for the original scales and for log-transformed scales (prefixed with an L). Correlations between the predictors and the dependent variable APD_{20} are illustrated in Fig. 5. Derived formulas for predicting APD_{20} by means of various transforms are given in Table VIII, which compares our "best" model with the model proposed by Cockroft et al.[7] who used histamine and a somewhat different method for quantitating skin sensitivity.

Results showed the following: (1) All models, single and multiple, fit better when the asthma and rhinitis subgroups are separated. The R^2 measure was usually low in the combined groups and substantially higher in most cases for individual asthma and rhinitis groups. In particular, MPD_{20} (and $LMPD_{20}$) was a much better predictor of APD_{20} (and $LAPD_{20}$) for the separated groups. (2) Different models were best for the two groups of subjects (Table VIII). For asthmatic subjects, log transforms of both the dependent and independent variables yield the best R^2. Subjects with rhinitis, however, did not require logarithmic transforms. For these subjects, MPD_{20} and MTSlope produced the best predictions of APD_{20} with an R^2 of 0.915.

DISCUSSION

The results of these studies confirm, extend, or question the findings and conclusions of other investigators and provide new information on the relationships of responses to methacholine and allergens in responders to both of these agents. In confirmation of other studies, methacholine distinguished a group of individuals with atopic asthma from groups of nonasthmatic individuals with atopic rhinitis and normal, nonatopic subjects. The use of dose-response slopes allowed comparisons of groups failing to generate a PD_{20} FEV_1 within established limits of dosing.[13, 14] Comparisons of responders with methacholine bronchoprovocation showed that the asthmatic group was statistically different from the nonasthmatic groups by being both more sensitive (lower PD_{20}) and more reactive (steeper slope). Asthma and rhinitis groups composed of responsive individuals failed to show differences, however, in responses to allergen bronchoprovocation. Fewer patients with rhinitis generated a PD_{20} to allergen inhalation than did asthmatic patients, and this resulted in significant differences in slopes when all allergen-challenged individuals were compared; when the comparison was limited to responsive individuals, no significant group differences were found in allergen-induced PD_{20} or in slopes; in fact, the two groups showed complete overlap of individual scores (Table V and Figs. 1 and 2).

Earlier studies have generally found small and insignificant differences between groups with asthma and those with rhinitis after allergen bronchoprovocation.[4, 15, 16] The relationship between hyperresponsiveness to nonspecific and specific bronchial reactivity has been variously reported as a direct relationship or lacking correlation.[15, 17-20] An initial question in the present studies was whether careful analysis of dose-response data after specific and nonspecific bronchoprovocation would reveal response characteristics unique to the asthmatic patient. Because we were particularly interested in qualitative differences in hyperresponsiveness, we focused on those subjects who had a directly measurable PD_{20} with inhalational challenge (i.e., methacholine and allergen responders). Previous studies have not used individual asthmatic and rhinitis subjects responsive to methacholine challenge to the extent we have to evaluate the degree and quality of responsiveness to allergen bronchoprovocation. Stevens and Vermeire[5] measured PD_{15} (dose causing 15% decrease in FEV_1) for histamine and allergens (house dust mite and grass pollen) to com-

TABLE VIIB. Regression analysis: predictor of APD_{20} and $LAPD_{20}$ (log-transformed variables)

	Predictors	Coefficients of determination (R^2) for $LAPD_{20}$ Asthma $n = 20$	Rhinitis $n = 8$	All subjects $n = 28$
Single	$LMPD_{20}$	0.42	0.67	0.24
	LMTSlope	0.48	0.60	0.28
	LWheal	0.33	0.01	0.21
Multiple	$LMPD_{20}$ + LMTSlope	0.48	0.67	0.28
	$LMPD_{20}$ + LWheal	0.68	0.67	0.59
	$LMPD_{20}$ + LMTSlope + LWheal	0.70	0.67	0.60

pare subjects with allergic asthma, subjects with allergic rhinitis, and patients with both conditions and found significant differences, as we did, between the groups in nonspecific bronchial sensitivity but not in sensitivity to allergen challenge (house dust mite and grass pollen). In contrast to our findings, however, there was no significant correlation between individual values of PD_{15} histamine and either PD_{15} mite or grass pollen in any of the groups. The authors suggested that although separate mechanisms were responsible for nonspecific and specific bronchial hyperresponsiveness, the presence of asthma in an allergic individual is essentially determined by a high degree of nonspecific bronchial hyperresponsiveness.[5] These statements seem contradictory, and the latter inconsistent with the finding that excessive responsiveness in asthmatic patients compared with patients with rhinitis was not demonstrable by allergen bronchoprovocation in either their study or ours. Rather, nonspecific bronchial hyperresponsiveness alone cannot explain the asthmatic response to inhaled allergen; patients with rhinitis with demonstrably less nonspecific bronchial responsiveness compared with asthmatic patients could not be differentiated from the same asthmatic patients by responsiveness to allergen bronchoprovocation. In addition, we found that responsiveness of subjects with rhinitis to allergen had a closer correlation with methacholine responsiveness than was true in asthmatic subjects, suggesting that responsiveness of asthmatic patients to allergen was determined to a lesser extent by nonspecific responsiveness ($R^2 = 0.54$) than for patients with rhinitis ($R^2 = 0.89$), because R^2 indicates the percent of variability in APD_{20} that is removed by knowledge of MPD_{20}.[12]

No previous information is available, to our knowledge, correlating early responsiveness to specific and nonspecific bronchoprovocation in patients with asthma and those with rhinitis with the percent fall in FEV_1 during subsequent late responses. We found that asthmatic patients demonstrating dual responses to allergen were more responsive to allergen bronchoprovocation during the immediate phase and therefore received less antigen, which makes unlikely any explanation of late responses based on excessive dosing. The presence and severity of the late response significantly differentiated the asthma and rhinitis groups with little overlap. This difference supports the notion that late responses may be important in the pathogenesis of clinical asthma.[21, 22] More asthmatic patients had late responses than did patients with rhinitis (68% vs 23%) and a significantly greater drop in FEV_1 during the late response was found in the asthma group (24%) compared with the rhinitis group (11%). Factors reported by others to be important in determining the development of late responses include high levels of specific IgE antibody,[6] the severity of the early response,[22] and the degree of responsiveness to methacholine or histamine[23]; our results are in agreement with these reports for asthma, but not for rhinitis subjects.

We have extended studies of slopes to compare complete and terminal slopes and the relationships of slopes with other parameters in asthma and rhinitis groups. Several techniques have been used for analysis of dose-response curves and have involved both single dose-response relationships (such as PD_{20}) and multiple dose-response relationships including assessment of threshold and slope, area under the curve, and analysis of actual data points.[24, 25] Orehek et al.[1] proposed a distinction between bronchial sensitivity and reactivity in the analysis of cumulative dose-response curves and found no significant correlation between them in responses to carbachol. Several studies subsequently used PD_{20} and regression lines to obtain maximal information from dose-response curves after specific or nonspecific bronchoprovocation. A dichotomy between PD_{20} and slopes has suggested that the evaluation of both may provide worthwhile information, although this point has been controversial.[26-28] A necessary mathematic connection exists for individual challenges. Cockcroft and Berscheid[26] evaluated dose-response slopes in histamine-responsive subjects and reported that determination of either the

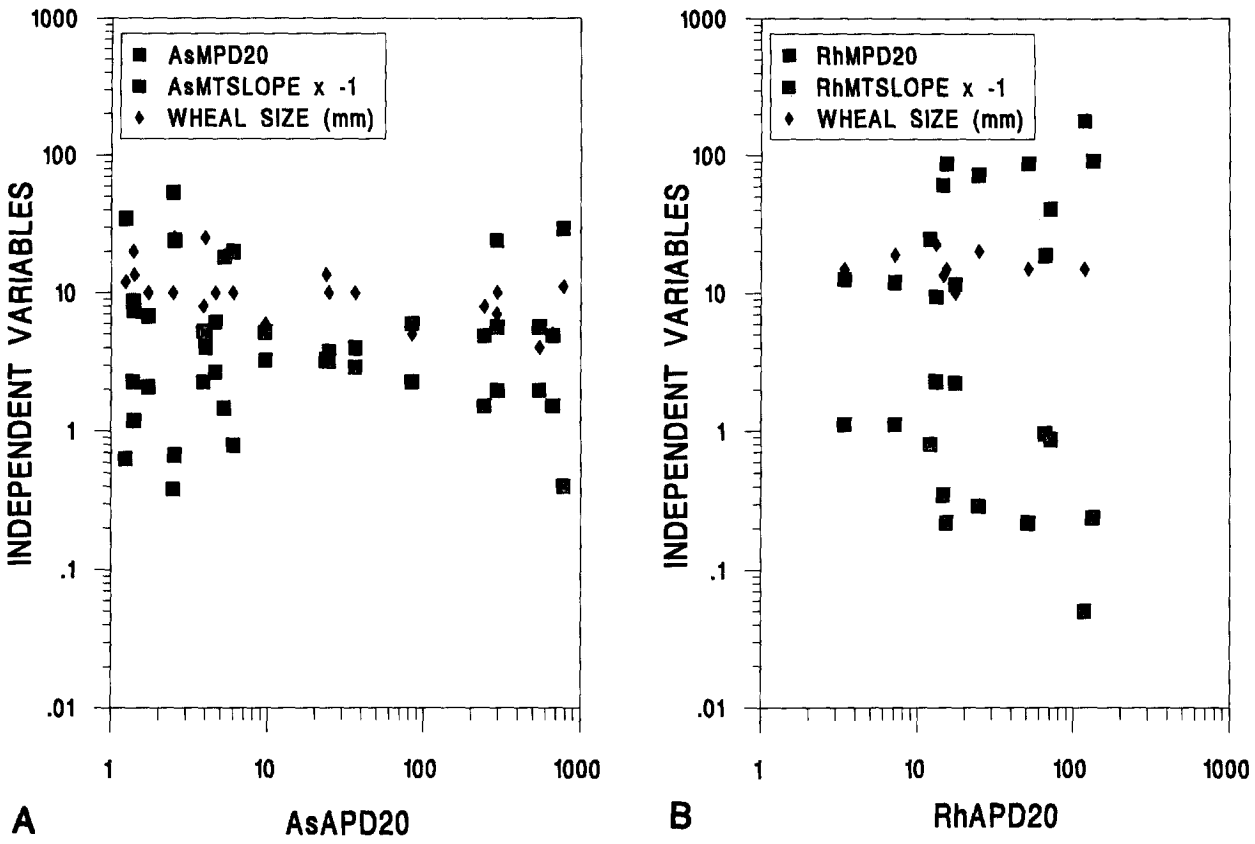

FIG. 5. Relationships for subjects listed in Table V between allergen PD_{20} FEV_1 (APD_{20}) and the three independent measures used in regression analysis: methacholine PD_{20} FEV_1 (MPD_{20}), methacholine terminal slope (MTSlope), and skin test wheal diameter. Untransformed values are plotted on log scales for illustrative purposes only; r_s and p are the same for linear and log-transformed data. **A.** Asthma subjects. r_s for APD_{20} versus MPD_{20} = 0.48 (p = 0.02); versus MTSlope = 0.67 (p = 0.001); versus wheal = −0.57 (p = 0.005). **B.** Rhinitis subjects. r_s for APD_{20} versus MPD_{20} = 0.65 (p = 0.02); versus MTSlope = 0.55 (p = 0.05); versus wheal = −0.18 (p = 0.64).

dose-response slope or the slope of log dose-response curve added little useful information to threshold concentration (PC_{20} FEV_1 or PC_{35} S_{GAW}). Woolcock et al.[29] on the other hand, in reviewing methodology for testing nonspecific bronchial hyperresponsiveness, recommended that the whole dose-response curve be reported with the FEV_1 plotted against log dose delivered. Another review of bronchial hyperresponsiveness was undertaken with the expressed purpose of propagating the usage of the shape of the dose-response curve as well as sensitivity, contending that hyperresponsiveness is a composite functional disorder including excessive airways narrowing as well as hypersensitivity.[28] In data generated but not shown in this report, individual dose-response lines determined by the principle of least squares showed excellent fit (averge $R^2 > 0.90$) when the percent fall in FEV_1 was plotted against untransformed CBUs for either methacholine or allergen. It was not unexpected, therefore, that dissection of the slope into the least squares fit from baseline to the point of 20% fall (not shown) and the terminal slope (TSlope), which connected the last two points on the curve, were almost identical to the entire slope (Slope) and behaved similarly in the various analyses made. The findings also suggested an early beginning of responses with our standard protocol and a minimal, if any, breakpoint in dose-response curves produced by standard bronchoprovocation sufficient to determine PD_{20}. In fact, a mild plateau effect was shown in asthmatic patients, patients with rhinitis, and normal responders with methacholine inhalation, in contrast to findings by others that asthmatic patients fail to plateau with increasing concentrations.[29]

An important question is whether evaluation of slopes added anything useful to analysis of bronchoprovocation data. The use of slopes made possible comparisons between larger groups of subjects within each category, since comparisons were not limited to responsive subjects, that is, those generating a PD_{20},

TABLE VIII. Predictive models for APD_{20} formulas derived by linear regression

	Cockcroft model[7]	Best model
Asthma $n = 20$	$LAPD_{20} = 1.29\ LMPD_{20} - 198$ Lwheal $- 2.58$ $R^2 = 0.61$	$LAPD_{20} = 1.32\ LMTSlope - 1.69\ Lwheal + 3.82$ $R^2 = 0.63$
Rhinitis $n = 8$	$LAPD_{20} = 0.64\ LMPD_{20} - 0.14$ Lwheal $+ 0.4$ $R^2 = 0.54$	$APD_{20} = 0.83\ MPD_{20} - 23\ MTSlope - 41.7$ $R^2 = 0.915$
All $n = 28$	$LAPD_{20} = 0.70\ LMPD_{20} - 2.32$ wheal $+ 3.12$ $R^2 = 0.49$	$LAPD_{20} = 0.72\ LMTSlope - 2.18\ Lwheal + 3.82$ $R^2 = 0.51$

and the contour of the slope (slope vs TSlope) could be evaluated. In addition, an apparent discrepancy was found between sensitivity and reactivity to allergen bronchoprovocation when the asthma and rhinitis groups were compared, in that asthmatic subjects were less sensitive but more reactive than subjects with rhinitis (Tables IV and V), true also when analysis was confined to ragweed challenge alone (data not shown). Although the differences did not reach clinical significance, they suggest that hyperreactivity might be worth further attention in studies of asthma pathogenesis. Finally, the addition of MTSlope to MPD_{20} as best predictors for APD_{20} did not change the coefficient of determination (R^2) in asthmatic subjects, but increased the R^2 from 0.89 to 0.97 in subjects with rhinitis, indicating that MTSlope contributes independently toward determination of APD_{20} in subjects with rhinitis, and the use of both leaves very little to be accounted for in determining APD_{20}. The use of both MPD_{20} and MTSlope in predicting APD_{20} in asthmatic patients, however, leaves 46% unaccounted for. Continued evaluation of slopes seems worthwhile for experimental purposes, but has little if any apparent clinical value at present except in the evaluation of populations.[13]

Various data transformations are regularly applied to dose-response variables as analyzed here. We have indicated elsewhere that such transformations are used for various reasons,[30] including the desire to stabilize or normalize error variances, to make linear a nonlinear relationship, or to satisfy a priori concepts, but that rote or traditional use of any particular data transformation may hinder scientific analysis of data and comparisons of various studies. The use of log-dose response plots has been customary, but Cockcroft and Berscheid[26] reported the slope of the dose-response curve appeared to fit the linear model better than the logarithmic model, with a better mean r^2, and this was also our finding. Log transformations of subsequent data for analysis gave variable results as illustrated in Tables VII and VIII and further substantiate the need for care in the use of logarithmic transformations. Computer programs allow multiple comparisons of transformed and untransformed data. Our use of nonparametric tests for between-group comparisons (Wilcoxon rank sum test) and correlations (Spearman's rank correlation), however, avoided any potential controversy concerning these analyses.

Cockcroft et al.[7] have proposed that allergen inhalation tests may be replaceable by determination of allergen sensitivity (skin tests or RAST) and nonspecific bronchoprovocation (methacholine or histamine) in predicting responses to inhaled allergen. We conclude from our findings, however, that (1) allergen responsiveness as measured by APD_{20} is determined largely by MPD_{20} in subjects with rhinitis but less so in asthmatic subjects; (2) the quantitation of skin test reactivity adds little to the equation (except to eliminate nonresponders), and (3) any formula derived to help predict allergen PD_{20} from methacholine data will not apply to both asthmatic patients and patients with rhinitis and may therefore have very limited practical value.

We also conclude that bronchial responsiveness to allergen bronchoprovocation in allergic individuals has little relationship to the presence or absence of clinical asthma caused by natural environmental challenge. This raises doubts about the validity and utility of laboratory bronchoprovocation with allergen and the significance of allergen-induced acute responses in the pathogenesis of asthma.[31-34] If asthmatic patients and patients with rhinitis respond similarly to inhaled allergen to which they have positive skin tests, why do only asthmatic patients respond in this way on natural exposure to allergen? Possible explanations include the following: (1) laboratory challenge is artifactual and allows deposition of allergen, which does not occur on natural exposure, with results that are essentially "a skin test of the lung" and irrelevant to clinical disease[32]; (2) only asthmatic patients inhale particulate environmental allergens in such a way as to allow deposition, absorption, or allergen handling sufficient to trigger bronchial responses; (3) late responses are primarily responsible for the development

of airways inflammation and clinical asthma, and, for whatever reason, occur more frequently and more severely in the asthma-prone than individuals with rhinitis[35]; and (4) mechanisms responsible for clinical asthma are to a considerable extent independent of early responses demonstrable in the laboratory to either allergen or methacholine and are absent in subjects with rhinitis.

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