

Factors contributing to the occurrence and predictability of bronchial hyperresponsiveness to methacholine in children

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Using a stepwise logistic regression analysis, we investigated clinical data, allergologic findings, spirometric data, and the cellular and humoral immune system in order to gain new insights into the role these parameters play in bronchial hyperresponsiveness to methacholine in children and to create a model for the prediction thereof. Bronchial hyperresponsiveness, which was found in 124 of 462 children (26.8%), was observed to have been influenced by an increased level of eosinophils, the positivity of the skin prick test for any of the allergens tested, a decreased baseline forced expiratory volume in 1 second (FEV₁) (percent predicted), a decreased maximum expiratory flow at 50% expiration as a percent of forced vital capacity, and a decreased level of κ -chain-assembled immunoglobulins. Logit analysis disclosed that the influence of all other parameters on the occurrence of bronchial hyperresponsiveness was of no further statistical significance. The degree of bronchial hyperresponsiveness (provocative dose causing a 20% fall in FEV₁) showed a statistically significant correlation with the eosinophil count (Spearman's $r = -0.198$) and FEV₁ (percent predicted) (Spearman's $r = 0.203$). Our findings suggest that allergic sensitization and eosinophilic reaction in children are major factors in contributing to the occurrence of bronchial hyperresponsiveness to methacholine. (J ALLERGY CLIN IMMUNOL 1994;93:735-42.)

Key words: Bronchial hyperresponsiveness, spirometry, allergy, immune system

Bronchial hyperresponsiveness is the key feature of bronchoobstructive disorders.¹⁻⁶ It may, however, be found not only in patients with current bronchial asthma but also in former asthmatics, in patients with allergic rhinitis or atopic dermatitis, and even in subjects who are free of symptoms.⁷⁻¹⁴ Increased bronchial hyperresponsiveness may occur after exposure to other different kinds of stimuli.¹⁵⁻¹⁸ Apart from allergens, infections, smoking, and certain air pollutants have been considered risk factors and triggers for increased bronchial hyperresponsiveness.^{15, 16, 19-24}

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Abbreviations used

CI:	Confidence interval
FEV ₁ :	Forced expiratory volume in 1 second
FITC:	Fluorescein isothiocyanate
FVC:	Forced vital capacity
MEF ₅₀ :	Maximum expiratory flow at 50% expiration
MEF ₅₀ /FVC%:	MEF ₅₀ as percent of FVC
PD ₂₀ :	Provocative dose causing a 20% fall in FEV ₁
PE:	Phycoerythrin

These causes have been suspected of inducing and aggravating inflammatory reactions in the lower respiratory tract.²⁵ A former investigation showed that eosinophils in particular are involved in the local inflammatory process.²⁶ In addition, interactions between the cellular immune system and bronchial responsiveness have been suspected.^{26, 27} Epidemiologic studies provided indefinite answers to all questions that arose in

connection with the relationships of bronchial hyperresponsiveness, airway inflammation, and the symptoms of asthma in children.

The objectives of this study were to investigate the factors that may have an impact on bronchial hyperresponsiveness to methacholine in children by undertaking a stepwise logistic regression analysis and to attempt to create a model through which bronchial hyperresponsiveness can be predicted.

METHODS

Test subjects and questionnaire

The target group consisted of 616 schoolchildren from all available classes of four randomly selected Austrian schools. Written informed consent from parents for children to participate in all steps of the study was obtained in 462 cases. These 462 children were analyzed for factors suspected of contributing to the development of bronchial hyperresponsiveness. The study was performed in fall, at the end of the pollen season. At the time of investigation, there was no sign of epidemic disease caused by viruses or other pathogens. A pediatric questionnaire (American Thoracic Society–Division of Lung Diseases–78-C)²⁸ on personal data and environmental conditions, which had been simplified in view of local necessities, was given to the parents by the teachers for joint evaluation.²⁸ In particular, we focused on the questions referring to “cough with colds” or “apart from colds” (American Thoracic Society–Division of Lung Diseases–78-C, questions 14A or 14B), “wheezing” (question 17), “breathlessness” (question 18A), and “susceptibility to chest colds” (question 16A).

Lung function and methacholine provocation

Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), and maximum expiratory flow at 50% expiration (MEF₅₀) were measured according to standardized methods by means of a pneumotachograph (Spirflow; Erich Jaeger GMBH & Co. KG, Würzburg, Germany), and the values thus obtained were related to the normal values for Austria.^{28, 29}

In all subjects the methacholine provocation test was performed by means of an inspiration-triggered dosimeter (Bronchoscreen, Jaeger) releasing 5 μ l of methacholine per inhalation during quiet breathing. The dosimeter output was controlled by weight control of nebulized solution and showed values between 95% and 105%. The methacholine concentrations used were 0.1% (28 inhalations; total dose, 140 μ g), 1% (28 inhalations; cumulative dose, 1540 μ g), and 5% (6 inhalations; cumulative dose, 3040 μ g). Spirometry was performed 3 minutes after each total dose of methacholine had been reached. The provocation test was considered to be positive if FEV₁ had declined by 20%. The provocative dose causing a 20% fall in FEV₁ (PD₂₀) was calculated according to the logarithmic-linear model.

Allergologic and immunologic tests

Skin prick testing was done according to standardized skin prick test methods by one experienced investigator with the following allergens:³⁰ *Dermatophagoides pteronyssinus* and *D. farinae*; grass, rye, birch, and mugwort pollen; cat, dog, and horse dander; *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species; negative control; and 1% histamine control (Pangramin; Abello, Madrid, Spain). The skin prick test result was considered to be positive if the wheals caused by any of the allergens exceeded those of the negative control by 2 mm in diameter.

Total IgE was determined by means of the Phadezym PRIST (Pharmacia, Uppsala, Sweden) method.^{31, 32} Specific IgE levels against house dust mite (*D. pteronyssinus*), grass pollen, and birch pollen were determined by the Phadezym RAST method. The finding was considered positive if specific IgE was above 0.34 Pharmacia RAST unit (PRU).

For each test subject, the white blood cell count and the differential cell count were determined. Lymphocyte subpopulations were examined by use of immunofluorescent staining with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies. Aliquots of 100 μ l of whole blood and 100 μ l of phosphate-buffered saline were incubated with 10 μ l of each of the following combinations of antibodies: OKT3(CD3)-FITC and OKB7(CD21)-PE, OKT4(CD4)-FITC and OKT8(CD8)-PE, OKNK(CD16)-FITC and OKDR-PE (Ortho Diagnostic Systems, Inc., Raritan, N.J.). The lymphocytes were identified by their light scattering properties. Immunofluorescence analysis was carried out on a cytofluorometer (Ortho-Cytoron, Ortho Diagnostic Systems, Inc.) after lysis of the red blood cells.³³ The gates for the fluorescence signals were set in accordance with the results of autofluorescence, nonspecific immunofluorescence of FITC- or PE-conjugated nonspecific mouse antibodies, and single green or single red immunofluorescent staining.

The humoral immune system (IgG, IgA, IgM, κ -chains, λ -chains, complement C3, complement C4) was investigated with the use of a Kallestad kinetic nephelometer (Kallestad, Chaska, Minn.).

Statistical methods

The influence of clinical findings and laboratory data on the dichotomous distribution of bronchial hyperresponsiveness was investigated by undertaking a forward stepwise logistic regression analysis with the maximum likelihood ratio method.³⁴ Independent categorical (binary) values are expressed as odds ratios with 95% confidence intervals (CIs); independent continuous values are expressed as means with 95% CIs. Probability (*p*) values less than 0.1 were entered into the equation. Further analyses of the relationship between the degree of bronchial hyperresponsiveness and other factors that had an impact on the occurrence of bronchial hyperresponsiveness were performed by use of Spearman's

TABLE I. Study subjects' data

	BHR ⁺ group (n = 124)		BHR ⁻ group (n = 338)	
Subjects (M/F)	82	(66.1%)/42 (33.9%)	188	(55.6%)/150 (44.4%)
Age (yr)	11.6	(11.4-11.8)	11.8	(11.7-11.9)
M/F	11.6	(11.4-11.8)/11.7 (11.4-12.0)	11.8	(11.7-11.9)/11.8 (11.6-12.0)
Cough	19	(15.3%)	19	(5.6%)
M/F	14	(17.1%)/5 (11.9%)	11	(5.9%)/8 (5.3%)
Wheezing	40	(32.3%)	69	(20.4%)
M/F	31	(37.8%)/9 (21.4%)	42	(22.3%)/27 (18.0%)
Breathlessness	22	(17.7%)	35	(10.4%)
M/F	18	(22.0%)/4 (9.5%)	21	(11.2%)/14 (9.3%)
Susceptibility to chest colds	39	(31.5%)	74	(21.9%)
M/F	25	(30.5%)/14 (33.3%)	38	(20.2%)/36 (24.0%)
Active smokers	7	(5.6%)	24	(7.1%)
M/F	4	(4.9%)/3 (7.1%)	13	(6.9%)/11 (7.3%)

Values are given as frequencies with percentages or as means with 95% CIs in parentheses.
BHR, Bronchial hyperresponsiveness.

TABLE II. Binary factors and their odds ratios for the prediction of bronchial hyperresponsiveness

Binary factors	Odds ratios (95% CI)	
	Unadjusted	Adjusted
Sex (M/F)	1.56 (1.01-2.41) <i>p</i> = 0.041	1.08 (0.66-1.75) <i>p</i> = 0.762
Cough	3.04 (1.53-6.03) <i>p</i> = 0.002	1.58 (0.74-3.39) <i>p</i> = 0.241
Wheezing	1.86 (1.16-2.97) <i>p</i> = 0.009	1.34 (0.80-2.23) <i>p</i> = 0.266
Breathlessness	1.87 (1.03-3.37) <i>p</i> = 0.038	1.14 (0.60-2.18) <i>p</i> = 0.688
Susceptibility to chest colds	1.64 (1.03-2.61) <i>p</i> = 0.037	1.43 (0.86-2.36) <i>p</i> = 0.167
Recent chest colds	1.04 (0.67-1.62) <i>p</i> = 0.852	0.90 (0.56-1.45) <i>p</i> = 0.662
Positive skin prick test result	2.93 (1.84-4.66) <i>p</i> < 0.0001	2.32 (1.41-3.82) <i>p</i> = 0.001
Positive RAST result	1.90 (1.20-2.99) <i>p</i> = 0.006	0.88 (0.45-1.70) <i>p</i> = 0.694
Manifest allergies	3.01 (1.75-5.19) <i>p</i> = 0.0001	1.13 (0.49-2.60) <i>p</i> = 0.774
Active smokers	0.78 (0.32-1.90) <i>p</i> = 0.573	0.98 (0.38-2.54) <i>p</i> = 0.961
Passive smoking	1.06 (0.70-1.65) <i>p</i> = 0.733	1.02 (0.65-1.62) <i>p</i> = 0.920

Factors with *p* values of less than 0.1 after adjustment are incorporated in the prediction model.

rank test.³⁵ Statistical significance was defined as *p* < 0.05.

RESULTS

Data from male and female test subjects are compared in Table I. With respect to clinical data (sex, age, weight, height, active or passive smok-

ing, and subjective complaints) no difference was seen between the 462 children who fully participated in the study and the remaining 154 children for whom only data of some parts of the study were available. Bronchial hyperresponsiveness was found more frequently in male children (Table II); subjective complaints (cough, wheez-

TABLE III. Anthropometric and spirometric data in the prediction of bronchial hyperresponsiveness

Continuous factors	BHR ⁺ group (n = 124)	BHR ⁻ group (n = 338)	Unadjusted		Adjusted	
			χ^2	p Value	χ^2	p Value
Age (yr)	11.6 (11.4-11.8)	11.8 (11.7-11.9)	1.61	0.204	1.32	0.250
Height (cm)	154 (152-155)	153 (152-155)	0.03	0.866	0.15	0.703
Weight (kg)	46 (44-48)	46 (45-47)	0.00	0.974	0.64	0.422
FVC (%pred)	96.5 (94.6-98.4)	99.3 (98.1-100.4)	6.37	0.012	0.17	0.677
FEV ₁ (%pred)	94.6 (92.5-96.8)	99.3 (98.2-100.4)	17.40	<0.0001	13.50	0.0002
FEV ₁ /FVC (%)	83.5 (82.5-84.4)	85.5 (85.0-86.1)	13.80	0.0002	0.05	0.826
MEF ₅₀ /FVC (%)	99.1 (95.4-102.7)	107.5 (105.4-109.6)	16.27	0.0001	6.01	0.014

Values are given as means with 95% CIs in parentheses.
Factors with *p* values of less than 0.1 after adjustment are incorporated in the prediction model.

ing, breathlessness, susceptibility to chest colds, and manifest allergies) were found more frequently in children with bronchial hyperresponsiveness. Sensitization to aeroallergens, which was identified by a positive skin prick test or RAST result, was seen more frequently in children with hyperresponsiveness. Boys had positive RAST results more frequently (31.5% vs 18.2%; *p* < 0.002) and positive skin prick test results more frequently (28.9% vs 15.6%; *p* < 0.001) than girls. As to aeroallergens, sensitization to grass pollen was seen most frequently (18.2% in RAST and 11.5% in prick test), followed by sensitization to house dust mite (11.5% in RAST and 11.3% in prick test), tree pollen (6.9% in RAST and 6.1% in prick test), weed pollen (8.7% in prick test), animal danders (5.2% in prick test), and molds (1.7% in prick test). There was no particular allergen that had a higher impact than another, nor was bronchial hyperresponsiveness influenced by wheal size or number. The odds ratios and 95% CI for positive skin prick test results and bronchial hyperresponsiveness were 2.34 (1.28-4.26) for grass pollen, 2.21 (1.21-4.06) for house dust mites, 4.02 (1.18-8.92) for tree pollen, 2.73 (1.40-5.35) for weed pollen, 2.03 (0.86-4.78) for animal dander, and 2.78 (0.67-11.63) for molds. The odds ratios (95% CI) for positive RAST and bronchial hyperresponsiveness were 1.79 (1.07-2.09) for grass pollen, 2.34 (1.28-4.26) for house dust mites, and 2.26 (1.07-4.77) for tree pollen. It was the presence and not the size of the wheals that showed the strongest correlation with RAST data.

The spirometric data were significantly lower in children with bronchial hyperresponsiveness (Table III). With regard to the immunologic data in the presence of bronchial hyperresponsiveness,

the levels of eosinophils and total IgE were found to be higher, whereas κ -chain-assembled immunoglobulins were seen in smaller quantities (Table IV). The degree of bronchial hyperresponsiveness (PD₂₀) in the 124 children concerned showed a statistically significant correlation with the eosinophil count (Spearman's *r* = -0.1978; *p* < 0.05) and baseline FEV₁ (percent predicted) (Spearman's *r* = 0.2028; *p* < 0.05). After the most significant factors (eosinophils, the value of predicted FEV₁, and the positivity of skin prick test) were entered into the logistic regression analysis step by step, all subjective complaints were not further predictive because they correlated closely with the parameters already entered. Total IgE correlated with the levels of eosinophils and the positivity of the skin prick test, and therefore it was of minor importance. In a further step MEF₅₀ as percent of FVC (MEF₅₀/FVC%) was entered, and this one together with the percentage of predicted FEV₁, which correlated with the other spirometric parameters, made these parameters unresponsive of bronchial hyperresponsiveness. Of the factors that had an impact on bronchial hyperresponsiveness, the last one entered into the stepwise logistic regression analysis was a low level of κ -chain-assembled immunoglobulins.

The statistical model for the prediction of bronchial hyperresponsiveness is given in Fig. 1. It allowed a highly significant prediction of bronchial hyperresponsiveness, when a probability at a terminus technicus above 0.25 was used. However, the sensitivity and the specificity were equal (about 63%), and the efficiency was low. With a probability at a cutoff point above 0.55, a decrease in sensitivity and an increase in specificity (97%) and efficiency (78%) were seen.

DISCUSSION

The object of our study was to examine the factors that may have an impact on the occurrence of bronchial hyperresponsiveness in children. The relatively high prevalence of bronchial hyperresponsiveness may be explained by higher concentrations and a relatively high total dose of methacholine used in the provocation test. As demonstrated by our result, bronchial hyperresponsiveness was strongly influenced by a high eosinophil level, the positivity of the skin prick test, and spirometric parameters. The elevated eosinophil count found in the peripheral blood of children with bronchial hyperresponsiveness indicates the role of these inflammatory cells. Previous studies have demonstrated the presence of eosinophilia in bronchoalveolar lavage fluid derived from patients with allergic asthma during the late-phase reaction.^{27, 36, 37} In these experiments eosinophilia has also been observed in peripheral blood as well. Accordingly, the increased levels of eosinophils in the peripheral blood of children with bronchial hyperresponsiveness, as seen in this study, may be interpreted as an indicator of inflammatory reactions.³⁸

The external factor that had the highest impact on the occurrence of bronchial hyperresponsiveness was the sensitization to a great variety of aeroallergens, which was detected by skin prick testing. Similarly, a study performed in children from various areas of Australia reported that it was the total load of aeroallergens and not the exposure to a particular allergen that was responsible for bronchial hyperresponsiveness.^{39, 40} Moreover, in special conditions specific allergens may also increase the risk for bronchial hyperresponsiveness.⁴¹⁻⁴³ Furthermore, we found that the presence of clinically manifest allergies, the total IgE levels, and the presence of allergen-specific IgE against the most common aeroallergens (house dust mite, grass pollen, and birch pollen) had a lower impact on bronchial hyperresponsiveness than the results of skin prick testing; therefore, the prediction of bronchial hyperresponsiveness by means of logistic regression analysis was not further influenced by these factors. The fact that the skin prick test result was positive to any of the allergens tested had a strong influence on bronchial hyperresponsiveness, which suggests the significance of allergies in this disorder.⁴⁴⁻⁴⁸ This also suggests a possibly frequent, subclinical sensitization to aeroallergens,⁴⁹ which may not have been clearly identified as an allergic disorder in

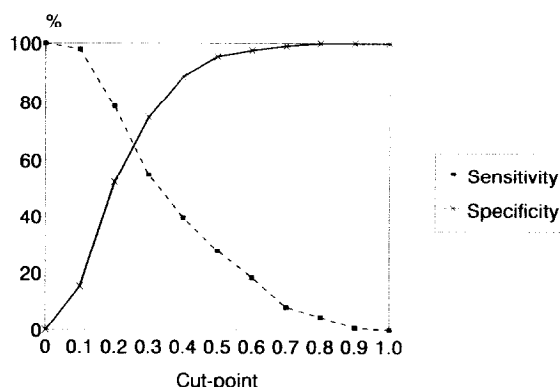


FIG. 1. Logistic model for the prediction of bronchial hyperresponsiveness: Probability of bronchial hyperresponsiveness = $\exp(4.27 + 0.1614x_1 - 0.04048x_2 + 0.843x_3 - 0.01422x_4 - 0.00078x_5) \div [1 + \exp(4.274 + 0.1614x_1 - 0.04048x_2 + 0.843x_3 - 0.01422x_4 - 0.00078x_5)]$. x_1 , Count of eosinophil granulocytes as percentage of the leukocyte count; x_2 , FEV₁ in percent predicted of the normal values; x_3 , positivity of the skin prick test to any of the allergens tested; x_4 , MEF₅₀/FVC %; x_5 , level of κ -chain-assembled immunoglobulins (mg/100 ml). A case is predicted to be in the group of children with bronchial hyperresponsiveness (*BHR*) if the probability is greater than that at a certain cutoff point. The higher the value of the cutoff point, the more the sensitivity for the prediction of bronchial hyperresponsiveness decreases and the specificity increases.

all children. Respiratory tract symptoms (cough, wheezing, breathlessness, or the susceptibility to chest colds) were reported more frequently by children with bronchial hyperresponsiveness, which is in agreement with several previous studies,⁵⁰ but our finding was that the occurrence of these symptoms depended on the presence of eosinophilia, preexistent airway narrowing, and the positivity of the skin prick test. The fact that the stepwise logistic regression analysis disclosed no other significant influence of reported symptoms on bronchial hyperresponsiveness furnishes further evidence for the low predictive value of subjective complaints in children.

As far as baseline spirometric data are concerned, we found that preexistent airway narrowing, demonstrated by a low FEV₁ (percent predicted) and MEF₅₀/FVC % may be considered a predictor of bronchial hyperresponsiveness.⁴⁸ Preexistent airway narrowing may correlate with bronchial asthma, even during asymptomatic intervals, but the high incidence of bronchial hyperresponsiveness precludes asthma as the only cause of bronchial hyperresponsiveness.

Of all parameters of the cellular and humoral

TABLE IV. Immunologic data in the prediction of bronchial hyperresponsiveness

Continuous factors	BHR ⁺ group (n = 124)		BHR ⁻ group (n = 338)		Unadjusted		Adjusted	
					χ^2	p Value	χ^2	p Value
Leukocytes	7200	(6860-7540)	7300	(7110-7490)	0.27	0.604	0.05	0.828
Lymphocytes (%)	42.3	(40.4-44.1)	41.1	(40.1-42.2)	1.19	0.276	0.57	0.451
Eosinophils (%)	4.7	(4.1-5.3)	2.9	(2.7-3.2)	28.43	<0.0001	19.23	<0.0001
CD3 ⁺	68.7	(67.4-70.1)	69.3	(68.6-70.0)	0.59	0.444	0.60	0.440
CD21 ⁺	8.9	(8.3-9.6)	8.7	(8.3-9.1)	0.24	0.628	0.50	0.480
CD4 ⁺	38.9	(37.8-39.9)	39.6	(38.8-40.3)	1.08	0.298	0.89	0.345
OKDR ⁺	13.4	(12.4-14.4)	12.8	(12.3-13.4)	1.08	0.298	2.65	0.103
CD8 ⁺	26.6	(25.6-27.6)	26.5	(25.9-27.1)	0.01	0.914	0.04	0.848
CD4 ⁺ /CD8 ⁺	1.55	(1.46-1.63)	1.59	(1.53-1.65)	0.60	0.440	0.42	0.519
CD16 ⁺	21.5	(20.3-22.7)	21.7	(21.0-22.3)	0.05	0.828	0.02	0.889
Total IgE (kU/L)	91	(66-116)	54	(43-65)	11.81	0.001	0.47	0.495
IgG (mg/100 ml)	1168	(1120-1217)	1219	(1181-1256)	2.15	0.143	2.14	0.144
IgA (mg/100 ml)	152	(133-170)	149	(141-157)	0.09	0.761	1.47	0.225
IgM (mg/100 ml)	119	(109-129)	128	(120-137)	1.84	0.175	0.00	0.994
κ -associated Ig (mg/100 ml)	868	(822-914)	941	(909-972)	6.20	0.013	3.17	0.075
λ -associated Ig (mg/100 ml)	490	(468-512)	529	(501-558)	3.23	0.072	0.49	0.482
κ/λ	1.84	(1.72-1.96)	1.91	(1.82-2.00)	0.83	0.363	0.00	0.958
Complement C3 (mg/100 ml)	154	(143-165)	156	(151-160)	0.09	0.758	0.61	0.434
Complement C4 (mg/100 ml)	30.9	(29.2-32.6)	32.0	(30.8-33.2)	0.97	0.324	0.09	0.762

Values are given as means with 95% CIs in parentheses. Factors with *p* values of less than 0.1 after adjustment are incorporated in the prediction model.

immune system, only low levels of κ -chain-assembled immunoglobulins had a low impact on the occurrence of bronchial hyperresponsiveness. The absence of major changes in the cellular and humoral immune system of children with this disorder underscores the hypothesis that immunodeficiencies may be irrelevant to bronchial hyperresponsiveness, and detectable alterations in the peripheral immune system, which are due to bronchial hyperresponsiveness, may defy measurement.^{26,27}

Measurement of bronchial hyperresponsiveness is often suggested; nevertheless, it is not very common to measure it in clinical practice.⁵¹ Therefore the attempt to predict bronchial hyperresponsiveness was made with the use of a model created on the basis of a stepwise logistic regression analysis. Despite the presence of many highly significant factors contributing to or correlating with it, bronchial hyperresponsiveness was not fully predictable. Our findings demonstrate that allergic sensitization in children and eosinophil

inflammatory reactions are strongly related to bronchial hyperresponsiveness to methacholine, even in a subclinical stage, and baseline spirometric data are significantly related to bronchial hyperresponsiveness. However, if bronchial hyperresponsiveness is suspected, no definite prediction can be made on the basis of contributing factors, and therefore the bronchial provocation test should be performed.

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ANNOUNCEMENT

**AMERICAN BOARD OF ALLERGY AND IMMUNOLOGY,
THE AMERICAN BOARD OF INTERNAL MEDICINE,
and THE AMERICAN BOARD OF PEDIATRICS**
are pleased to announce

CERTIFICATION IN CLINICAL AND LABORATORY IMMUNOLOGY

Examination Date: Monday, October 10, 1994
St. Louis, Missouri

Registration: through April 30, 1994

Completed applications must be postmarked by April 30, 1994. A nonrefundable late fee will apply to those applications received after the close of registration and before the cancellation deadline of August 1, 1994.

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