

Impaired beta-adrenoceptor function, increased leukocyte respiratory burst, and bronchial hyperresponsiveness

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Inflammatory processes have potential importance in the pathogenesis of bronchial hyperresponsiveness and asthma. Because beta-adrenoceptor function may be impaired in asthma, we studied regulation of the leukocyte respiratory burst using blood samples from subjects with bronchial hyperresponsiveness to methacholine. Leukocytes from hyperresponsive subjects were less responsive to the beta-agonist isoproterenol than were leukocytes from healthy control subjects. The magnitude of the respiratory burst was increased in cells from hyperresponsive subjects and correlated with the degree of methacholine responsiveness. These results demonstrate that peripheral leukocytes reflect a functional impairment in beta-adrenergic responsiveness that parallels airway hyperresponsiveness. Because untreated subjects demonstrated a reduction in beta-adrenergic response, the impairment in beta-adrenoceptor function was not a result of drug therapy and may be associated with the pathogenesis of asthma. (J ALLERGY CLIN IMMUNOL 1992;90:825-32.)

Key words: Leukocyte, beta-adrenoceptor, bronchial hyperresponsiveness, respiratory burst

Multiple studies have suggested that inflammatory processes are important in the pathogenesis of asthma.¹⁻³ In particular, inflammatory cells infiltrate the lungs during an asthmatic exacerbation.^{4, 5} Mediators generated by these cells including platelet-activating factor, leukotrienes, and superoxide anion can contribute to bronchial hyperresponsiveness.⁶⁻⁸ Pharmacologic intervention to prevent inappropriate inflammatory responses could have major therapeutic importance.

Although the importance of beta-adrenoceptor ag-

Abbreviations used

FMLP:	N-formyl-methionyl-leucyl-phenylalanine
FEF ₅₀ :	Forced expiratory flow at 50%
SFEF ₅₀ :	Slope of the percent decrease in FEV ₅₀ induced by methacholine (per milligram)
PD ₃₅ FEF ₅₀ :	Provocative dose of methacholine inducing 35% fall in FEF ₅₀
FEV ₁ :	Forced expiratory volume in 1 second
PMN:	Polymorphonuclear neutrophil

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onists in the treatment of asthma is well established, effects of these agents on inflammatory cell function in asthmatic patients are not well understood. Beta-adrenoceptor agonists inhibit leukocyte responses.^{9, 10} Consequently, beta-adrenoceptor agonists including plasma epinephrine could be important in the regulation of inflammatory mediator release. Because beta-adrenoceptor function may be abnormal in asthma,^{11, 12} impaired beta-adrenoceptor regulation of inflammatory responses may contribute to the pathogenesis of asthma.

To determine whether beta-adrenergic regulation of leukocytes is altered in asthma, we have evaluated the leukocyte respiratory burst with phlebotomy samples from subjects with bronchial hyperresponsiveness.

During the respiratory burst, a principal bactericidal response,¹³ superoxide anion and other toxic oxygen metabolites are generated. Because superoxide anion generation is regulated by very low concentrations of beta-adrenoceptor agonists,^{9, 14} evaluation of beta-agonist-mediated inhibition of the respiratory burst provides a sensitive measure of beta-adrenoceptor function that may be relevant to the pathogenesis of asthma.

MATERIAL AND METHODS

Subjects

Subjects who were healthy with no history of respiratory or atopic disease and subjects who had a history of intermittent wheezing suggesting bronchial hyperresponsiveness were invited to participate in the study, which included measurements of pulmonary function and methacholine responsiveness. Subjects were 49 ± 20 years of age (mean $\pm$ SD). All volunteers were screened with a medical history, physical examination, and ECG. Subjects with a history of cognitive impairment, pregnancy, ischemic heart disease, thyroid disorder, smoking (current or more than 10 pack years history), or therapy with beta-blockers, calcium channel blockers, or angiotensin-converting enzyme inhibitors were excluded. Patients with recent (within 6 weeks) respiratory tract infection, change in bronchodilator medications, or antihistamine treatment were also excluded. No subjects had used oral corticosteroids for at least 3 months. Oral bronchodilator medications were withheld for 48 hours, and inhalers were withheld for 12 hours before the study. Three subjects had used inhaled beclomethasone, four had used inhaled cromolyn, and four had used ipratropium. Because patients with severe airway obstruction could not withhold medications for a sufficient period of time, the study was limited to subjects with mild symptoms. All subjects refrained from consuming foods or drinks containing caffeine and they used no oral medications for 24 hours before the study. Study conditions were approved by the University of Washington Human Subjects Committee.

Methacholine challenge

Subjects who potentially would have nonspecific bronchial hyperresponsiveness were identified by a history of intermittent seasonal or exercise-induced wheezing. Subjects selected as potentially hyperresponsive had 20% variability in forced expiratory volume in 1 second (FEV₁). All subjects had a baseline FEV₁ of greater than or equal to 60% predicted. Twenty-seven potentially hyperresponsive and 28 subjects unlikely to be hyperresponsive were identified.

Methacholine challenge was administered by the modified Newcastle dosimeter method,¹⁵ with an initial 1.5 μ g dose and then doubling cumulative doses at 5-minute intervals until a 35% fall in the forced expiratory flow at 50% of vital capacity (FEF₅₀) was achieved or 6.4 mg dose of methacholine chloride was administered. Single FEF₅₀ measurements were performed before each additional metha-

choline dose. Because a number of subjects received the maximal 6.4 mg dose of methacholine before a 35% fall in FEF₅₀ was achieved, the PD₃₅ FEF₅₀ (provocative dose of methacholine inducing 35% fall in FEF₅₀) did not provide an optimal measure to quantify bronchial responsiveness. The log of the simplified slope of the decrease in FEF₅₀ (percent decrease in FEF₅₀ induced per milligram methacholine (SFEF₅₀)) was calculated and used as a measure of each subject's nonspecific bronchial responsiveness. Although parametric analysis was used in most studies, a hyperresponsive group ($n = 26$) was classified by a log SFEF₅₀ greater than one standard deviation over the mean of the group who had no history of wheezing.

Measurement of leukocyte respiratory burst

To minimize artifacts from in vitro manipulation of cells, the respiratory burst was measured at 37° C with a dilution of whole blood within 5 minutes of the time of phlebotomy. A 185 ml blood sample was obtained from each subject after 30 minutes of supine rest and immediately before pulmonary function testing (and before methacholine challenge). The samples were then divided and aliquots used for measurement of plasma norepinephrine and epinephrine (radioenzymatic assay), IgE (amplified immunoradiometric assay; Diagnostic Products Corporation, Los Angeles, Calif.), lymphocyte beta-adrenoceptor binding, and the leukocyte respiratory burst. The aliquot used to measure the respiratory burst was immediately diluted 1:100 in phosphate-buffered saline solution, which included 100 mg/dl glucose and 1 mmol/L calcium. The blood dilution was automatically introduced into sample tubes containing appropriate concentrations of calcium ionophore (A23187) and isoproterenol. Luminol (1 μ mol/L) was included to detect superoxide anion and other oxygen metabolite generation. The luminescence was detected by use of a Packard (Packard Instrument Co., Downers Grove, Ill.) Picolight luminometer at 2-minute intervals, with samples maintained at 37° C. The respiratory burst as measured included responses from all blood elements including monocytes and eosinophils. Because of the numeric predominance of neutrophils with respect to other cells capable of generating oxygen metabolites, it is probable that measurements were most associated with the neutrophil respiratory burst.

Lymphocyte beta-adrenoceptor binding

Beta-adrenoceptor density and affinity were evaluated with lymphocyte membranes. Lymphocytes were isolated with Ficoll-Hypaque density gradient centrifugation and then lysed with 5 mmol/L TRIS HCl at 4° C. HEPES assay buffer (10 mmol/L HEPES, 5 mmol/L MgCl, 0.5 mmol/L sucrose, pH 7.4) was added and membranes fragmented by dounce. The membranes were isolated with ultracentrifugation and stored at -70° C until receptor analysis. Receptors were analyzed by use of 50 μ l aliquots of the membrane preparation (1×10^6 cells) with (iodine 125 [¹²⁵I]) iodocyanopindolol. Nine isotope dilutions were evaluated, and 5 μ mol/L propranolol was used to assess nonspecific binding. After incubation, membranes were vacuum filtered at 4° C through Whatman (Whatman Labsales, Hills-

boro, Ore.) GF/C filters (presoaked in polyethylenimine) with a Brandel (Brandel, Gaithersburg, Md.) cell harvester. Dried filters were cut and measured in an LKB (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.) 1281-802 gamma counter. Total counts were determined from 50 μ l aliquots at each isotope concentration. To determine receptor affinity, competition curves were determined by use of 25 membrane samples incubated with (^{125} I) iodocyanopindolol at the determined K_d with increasing isoproterenol concentrations (1 μ mol/L to 250 μ mol/L).

Statistics and curve fitting

Nonlinear curve fitting was performed with computer assistance by use of the Marquardt algorithm. Receptor density and antagonist affinity were determined from saturation isotherms calculated with use of Lunden I (Lunden software, Cleveland, Ohio). Agonist competition curves were evaluated with InPlot (Graphpad Software, San Diego, Calif.). Multiple regression, analysis of variance (ANOVA), and Student's *t* tests were performed with EcStatic software (Montpelier, Vt.).

RESULTS

To determine whether beta-adrenergic regulation of leukocyte function is impaired in subjects with bronchial hyperresponsiveness, leukocyte inhibition by the beta-agonist isoproterenol was evaluated in parallel with methacholine challenge. Isoproterenol induced significantly less maximal inhibition of the leukocyte respiratory burst in subjects with bronchial hyperresponsiveness (Fig. 1, ANOVA $F = 5.03$, $df = 1, 53$, $p < 0.05$). Inhibition of the respiratory burst induced by beta-adrenoceptor agonist was calculated relative to the magnitude of the respiratory burst induced in simultaneously activated control cells without isoproterenol. Leukocytes were activated with the calcium ionophore A23187, a means of cell activation that is very sensitive to inhibition by the beta-adrenoceptor agonist isoproterenol.¹⁴ The absolute magnitude of the respiratory burst induced by A23187 was not statistically different between hyperresponsive and normal subjects (2442 ± 201 and 2570 ± 221 , respectively, luminescence units reflect photomultiplier tube counts per second).

Beta-adrenoceptor binding studies were performed in parallel with polymorphonuclear neutrophil studies with aliquots of the same blood samples. Lymphocytes from subjects with bronchial hyperresponsiveness had a reduced percentage of high-affinity beta-adrenoceptors compared with those from control subjects (Table I). The number of receptors was not different between hyperresponsive and control subjects (Table I). Thus maximal inhibition of the leukocyte respiratory burst by isoproterenol and the percentage of lymphocyte high-affinity receptors were both decreased in association with bronchial hyperresponsiveness.

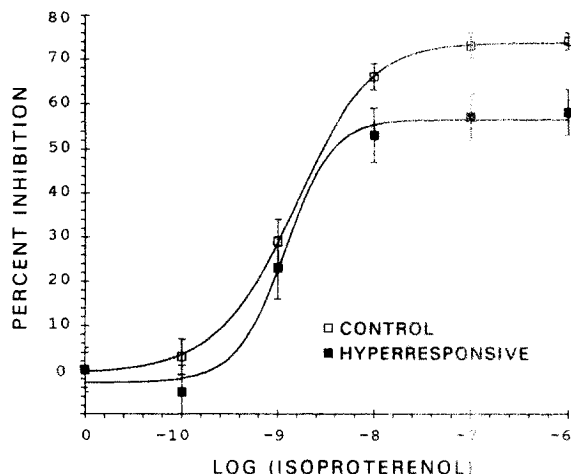


FIG. 1. Isoproterenol-induced inhibition of the respiratory burst in a dilution of whole blood detected with luminol after 0.2 μ mol/L A23187 stimulation. Inhibition was calculated as the percentage decrease compared with a sample simultaneously stimulated with A23187 in the absence of beta-agonist. Data shown are the mean $\pm$ SEM of samples from 29 subjects with low levels of bronchial responsiveness and 26 hyperresponsive subjects.

The maximal percent inhibition of the respiratory burst achieved with high concentrations of isoproterenol (100 nmol/L to 1 μ mol/L plateau) in vitro is shown as a function of response to methacholine (percentage decrease FEF_{50} per milligram of methacholine, $SFEF_{50}$) in Fig. 2, A. The ability of beta-agonist to inhibit the respiratory burst was markedly reduced in subjects with the most severe bronchial responsiveness to methacholine. Regression analysis demonstrated a significant linear correlation ($r = -0.47$, $p = 0.0001$). As demonstrated in Figs. 2, B and C, subjects with the greatest bronchial responsiveness also had a reduced percentage of high-affinity receptors and reduced high-affinity receptor K_d . Similar to the data for the respiratory burst, linear regression analysis demonstrated significant correlations between bronchial responsiveness and receptor characteristics ($r = -0.51$, $p = 0.0001$ and $r = -0.38$, $p = 0.001$ for Figs. 2, B and C, respectively). In contrast, receptor numbers did not appear to be correlated with bronchial responsiveness ($r = -0.04$, $p > 0.05$).

Although all patients withheld medications including inhalers for at least 12 hours, beta-adrenoceptor agonists orally or by inhalation are well known to cause beta-adrenoceptor desensitization.^{16, 17} Therefore, analysis was performed with results of studies in a subset of subjects who had received no bronchodilators (oral or inhaled beta-agonists) (Fig. 3). Although the number of subjects and range of bronchial

TABLE I. Measurements performed in control and hyperresponsive subject groups

	Control	Hyperresponsive	
Subjects	29	26	
SFEF ₅₀ (% decrease/mg)	34 ± 9	7195 ± 1618	p < 0.0001
FEV ₁ (L)	3.5 ± 0.15	2.6 ± 0.20	p = 0.0001
Epinephrine (pg/ml)	36 ± 3.4	31 ± 3.8	(NS)
Norepinephrine (pg/ml)	325 ± 21	398 ± 39	(NS)
IgE (U/ml)	42 ± 12	139 ± 45	p = 0.04
High-affinity receptors (%)	54 ± 3.3	35 ± 3.6	p = 0.001
High-affinity kd (nM)	251 ± 53	136 ± 27	p = 0.06
Low-affinity kd (μM)	8.3 ± 2.4	4.8 ± 2.1	(NS)
Receptors (BMax, fmol/mg)	35 ± 3.8	39 ± 3.6	(NS)
A23187 stimulated O ₂ ⁻	2570 ± 221	2442 ± 201	(NS)
Inhibition (percent control)			
1 nmol/L isoproterenol	29 ± 28	23 ± 38	(NS)
10 nmol/L isoproterenol	65 ± 18	53 ± 32	p = 0.09
100 nmol/L isoproterenol	73 ± 14	57 ± 27	p = 0.01
1 μmol/L isoproterenol	74 ± 12	59 ± 26	p = 0.006

Data indicate mean ± SEM.

TABLE II. Dependence of bronchial responsiveness (log [SFEF₅₀]) on other variables with use of multiple regression analysis; significance of improvement in regression model with inclusion of each variable

Variable	t score	p
Isoproterenol inhibition	4.14	0.001
High-affinity receptor (percent)	3.28	0.001
Subject age	2.92	0.003
Plasma IgE	2.10	0.036

Correlation: $r^2 = 0.54$.

ANOVA: $F = 15.2$; $df = 4,50$; $p < 0.0001$.

responsiveness were reduced, a correlation that was statistically significant could be identified between bronchial responsiveness and both beta-adrenergic inhibition of the respiratory burst ($r = -0.28$, $p < 0.05$) and decreased high-affinity receptors ($r = -0.46$, $p < 0.05$). Thus impaired beta-adrenergic response may be associated with bronchial hyperresponsiveness independent of medical therapy.

To determine which factors may be associated with increased bronchial responsiveness, a multiple regression model of bronchial responsiveness was evaluated, including beta-adrenoceptor characteristics, magnitude of the respiratory burst, age, plasma catecholamines, and IgE. Inclusion of age, IgE, lymphocyte high-affinity beta-adrenoceptors, and beta-adrenergic

inhibition of the respiratory burst (but not plasma epinephrine or norepinephrine) in the multiple regression model significantly ($p < 0.05$) reduced the unexplained variation in bronchial responsiveness (Table II). Because the regression model was improved by each of these variables independently, the physiologic characteristics represented by age, IgE, percentage of high-affinity receptors, and maximal achievable beta-adrenergic inhibition of the respiratory burst, each potentially contributed to the mechanisms of bronchial hyperresponsiveness.

Since the regression model fit was improved by both the percentage of high-affinity beta-adrenoceptors and the degree of beta-adrenergic inhibition of the respiratory burst, these variables did not appear to be simply different measures of the same physiologic characteristic. Regression analysis demonstrated that subjects with a low percentage of high-affinity receptors were not necessarily the same subjects who had impaired beta-adrenergic inhibition of the respiratory burst (linear regression, $r = 0.09$, $p > 0.05$, Fig. 4). Consequently, receptor binding characteristics and cellular effects of beta-adrenoceptor agonists may be at least partially independent characteristics of altered beta-adrenoceptor function.

The magnitude of the respiratory burst induced by A23187 (in the absence of isoproterenol) was reduced in subjects with the highest percentages of high-affinity receptors. Although regression analysis showed a significant correlation ($r = -0.32$, $p = 0.007$), the effect was principally apparent in subjects with more than 75% high-affinity receptors (Fig. 5). Within this small subset of subjects ($n = 6$) the magnitude of the

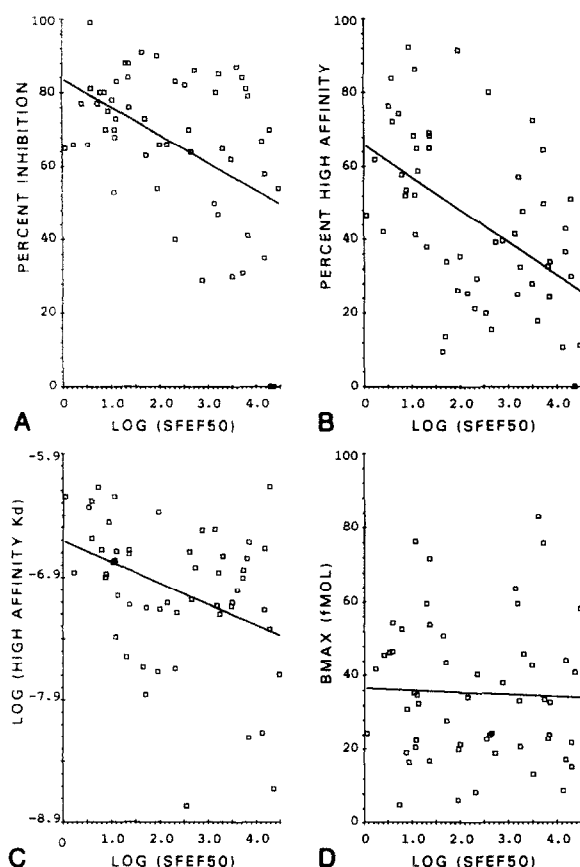


FIG. 2. Relationship of bronchial responsiveness (methacholine) to beta-adrenoceptor function as indicated by isoproterenol inhibition of respiratory burst (*panel A, top left*), lymphocyte beta-receptors in high-affinity state (*panel B, top right*), lymphocyte high-affinity receptor kd (*panel C, bottom left*), and lymphocyte receptors (*panel D, bottom right*). Regression analysis indicated that linear correlation shown was statistically significant ($p < 0.05$) between bronchial responsiveness and inhibition of the respiratory burst ($r = -0.47$), percent high-affinity receptors ($r = -0.51$), and high-affinity receptor kd ($r = -0.38$), but not receptor number ($r = -0.04$).

respiratory burst was highly correlated with plasma norepinephrine ($r = -0.89$, $p = 0.007$) suggesting that catecholamines at plasma concentrations may, in some subjects, have had an inhibitory effect on inflammatory response.

Studies of leukocyte regulation by beta-adrenoceptor agonists were performed with calcium ionophore activation. Because the magnitude of the respiratory burst induced by calcium ionophore was not altered in subjects with bronchial hyperresponsiveness, evaluation of relative beta-adrenergic effects was facilitated. However, leukocyte priming, which enhances cell activation by agents that require usual pathways of signal transduction, may not be reflected by calcium

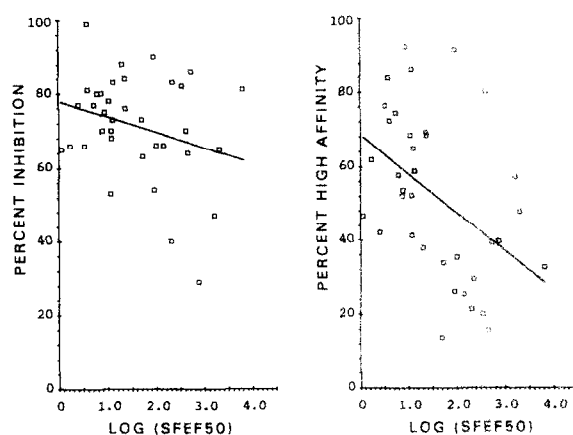


FIG. 3. Subset of subjects ($n = 36$) not exposed to oral or inhaled bronchodilators. Relationship of bronchial responsiveness (methacholine) to beta-adrenoceptor function as indicated by isoproterenol inhibition of respiratory burst (*panel A, left*) and lymphocyte beta-receptors in high-affinity state (*panel B, right*). Regression analysis demonstrated that linear correlation shown was statistically significant between bronchial responsiveness and inhibition of the respiratory burst ($r = -0.28$) and percentage of high affinity receptors ($r = -0.46$).

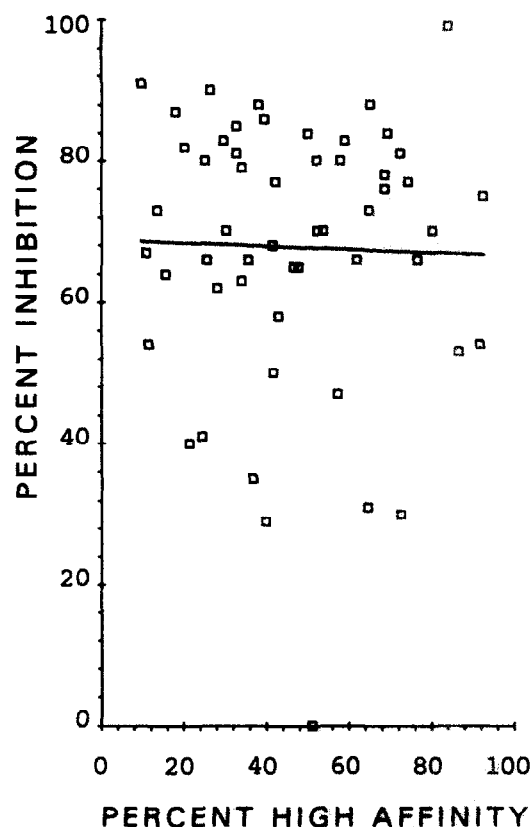


FIG. 4. Relationship between isoproterenol inhibition of the respiratory burst and lymphocyte high-affinity receptors. No statistically significant correlation was identified.

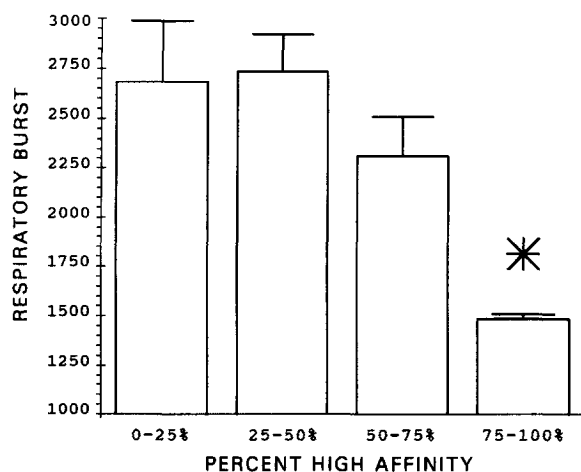


FIG. 5. Decrease in respiratory burst associated with increased percentage of high-affinity receptors. Respiratory burst was induced by 0.2 $\mu\text{mol/L}$ A23187 with a dilution of whole blood. High-affinity receptors measured on lymphocytes. Data are shown as mean $\pm$ SEM with samples from 13, 21, 17, and 6 subjects with 0% to 25%, 25% to 50%, 50% to 75%, and 75% to 100% high-affinity receptors, respectively. (* $p < 0.05$).

ionophore stimulation. Therefore activation of the respiratory burst by chemotactic peptide was also evaluated.

PMN activation by FMLP was increased in subjects with bronchial hyperresponsiveness (Fig. 6), and the respiratory burst magnitude was correlated with the degree of methacholine responsiveness. Multiple regression analysis revealed that increased magnitude of respiratory burst induced by FMLP was associated with either increased bronchial responsiveness ($p < 0.05$) or elevated plasma IgE (borderline significance, $p = 0.08$), but not isoproterenol inhibition of the respiratory burst, lymphocyte beta-adrenoceptor characteristics, plasma catecholamines, or age. Inclusion of both plasma IgE and bronchial responsiveness did not further improve the model, suggesting that these variables may be partially reflecting a common physiologic process.

DISCUSSION

This study demonstrates an association of bronchial hyperresponsiveness with both impaired beta-adrenergic function and increased peripheral leukocyte activation. The results are particularly important because all subjects were studied in the absence of bronchodilator medications. Consequently, our observations suggest that impaired beta-adrenergic responses may be related to disease pathogenesis.

Impaired beta-adrenergic response has been clearly demonstrated to occur in asthmatic patients.^{12, 16, 18, 19}

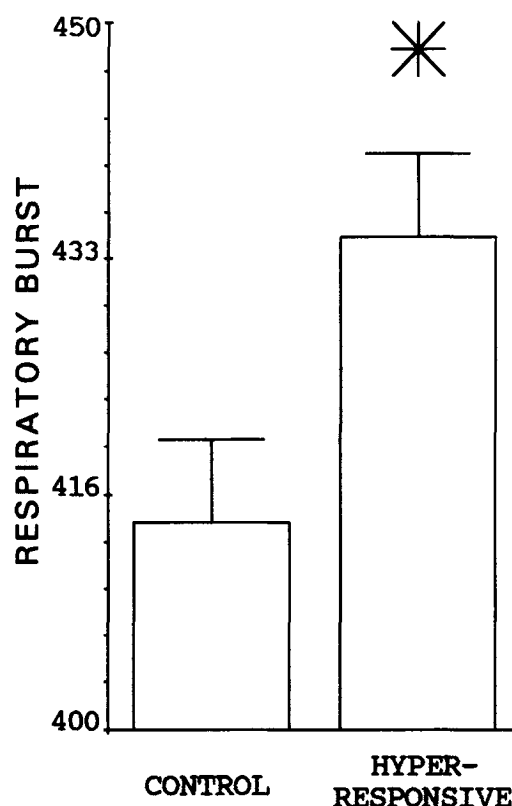


FIG. 6. Increased chemotactic peptide (FMLP) induced respiratory burst in subjects with bronchial hyperresponsiveness as defined with methacholine challenge. Data shown are mean $\pm$ SEM of samples from 29 subjects with low levels of bronchial responsiveness and 26 hyperresponsive subjects (* $p < 0.05$).

Because therapeutic beta-agonist administration rapidly causes receptor desensitization it has remained unclear whether beta-adrenoceptor changes are associated with the disease process or are a result of therapeutic intervention.^{12, 16} In the absence of drug exposure, impaired beta-adrenergic response has been demonstrated in atopic subjects.²⁰ However, few studies have identified impaired beta-adrenergic response in drug-free asthmatic subjects, and when such an association was observed, measures of beta-adrenoceptor response did not uniformly correlate with bronchial hyperresponsiveness.¹⁸ Our studies were unique in that subjects with mild bronchial hyperresponsiveness were studied and beta-adrenergic modulation of the respiratory burst, an extremely sensitive and physiologically relevant assay of beta-adrenergic response, was evaluated.

Our results clearly demonstrate a decrease in both beta-adrenergic response and high-affinity receptors in association with increased bronchial response to methacholine challenge. Because all subjects had abstained from medications for at least 12 hours and the

association of bronchial hyperresponsiveness with impaired beta-adrenoceptor function was further confirmed in the subset of subjects with no exposure to bronchodilators, beta-adrenoceptor agonist induced receptor desensitization was not responsible for the association.

Beta-adrenoceptor binding characteristics have been evaluated in most past studies of asthma. This study is relatively unique in that both beta-adrenoceptor binding characteristics and beta-adrenergic responses were evaluated in parallel with bronchial challenge. Although abnormalities of both beta-adrenoceptor binding and beta-adrenergic response were identified in association with bronchial hyperresponsiveness, the two measures of beta-receptors were not significantly associated (Fig. 4). The disparity may be a consequence of evaluation with different cell populations or the fact that reduced high-affinity receptor binding and impaired beta-agonist response are not the same physiologic phenomenon. Because both high-affinity receptors were reduced and beta-adrenergic regulation of leukocyte function was impaired, our results suggest that impaired adrenergic function in association with bronchial hyperresponsiveness may be multifactorial in origin. Such a conclusion is compatible with previous work, suggesting alterations at multiple sites including receptor binding,^{19, 21} cyclic adenosine monophosphate accumulation,²² and phosphodiesterase activity in association with asthma.²³

Asthma associated increases in plasma chemotactic factors, peripheral leukocyte priming, and enhanced leukocyte activation, particularly in association with asthmatic exacerbations, have all been well described.²⁴⁻²⁶ Our results demonstrate that enhanced leukocyte activation occurs not only during an exacerbation of asthma but also in subjects with mild bronchial hyperresponsiveness to methacholine. Furthermore, the degree of hyperresponsiveness correlates with the magnitude of the enhanced respiratory burst when induced by chemotactic peptide. Because the chemotactic peptide but not calcium ionophore induction of the respiratory burst was increased in association with bronchial hyperresponsiveness, the mechanisms of PMN stimulus-response coupling and regulation of specific signal transduction pathways may be important. Our observed increase in the respiratory burst indicates an enhanced reactivity of peripheral inflammatory cells that may contribute to bronchial hyperresponsiveness through the release of multiple inflammatory mediators.²⁷⁻²⁹

The possible relationship between enhanced inflammatory responses and impaired beta-adrenergic response in asthma is not well understood. Our results clearly demonstrate that both increased inflammatory

responses and decreased beta-adrenergic responses are associated with bronchial hyperresponsiveness to methacholine. The respiratory burst was less effectively inhibited by beta-agonists in hyperresponsive subjects. Thus it is plausible that an impairment in leukocyte regulation may contribute to enhanced inflammatory mediator release in asthma.

In conclusion, our results demonstrate that untreated subjects have an impairment in beta-adrenoceptor response that correlates with increased bronchial response to methacholine, and that bronchial hyperresponsiveness is associated with an enhanced peripheral leukocyte respiratory burst.

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Registration: through March 31, 1993

Completed applications must be postmarked by March 31, 1993, to avoid a late, nonrefundable penalty fee. No applications will be accepted after August 15, 1993.

Please address all requests to Herbert C. Mansmann, Jr., MD, American Board of Allergy and Immunology, 3624 Market St., Philadelphia, PA 19104-2675; phone 215/349-9466, FAX 215/222-8669.

Recertification is available to ABAI Diplomates via an Alternative Pathway of the 1993 Certification Process. Inquiries should be directed as noted.