Prednisone Pretreatment Leads to Histaminic Airway *Hypo*reactivity Soon after Resolution of the Immediate Allergic Response*

Dan R. Steinberg, M.D.;† David I. Bernstein, M.D.;‡
I. Leonard Bernstein, M.D.;§ and Christopher G. Murlas, M.D., F.C.C.P.||

We assessed the effect of prednisone pretreatment (50 mg/day for three days) on the development of the early increase in histamine reactivity that occurs soon after resolution of the immediate response in allergic humans. Four allergic subjects who were known to develop only isolated immediate responses upon Kentucky bluegrass inhalation, as well as four mild allergic asthmatic subjects known to develop typical dual phase responses, were evaluated. All testing was done more than nine weeks after the grass pollen season had ended. Allergen inhalation produced an immediate response in all subjects. However, upon resolution of the immediate response to allergen in these pretreated subjects, the PC_{200}His in all dual responding asthmatics and in three of the four isolated immediate responders had substantially increased above baseline values. We conclude that prednisone pretreatment leads to histaminic *hypo*reactivity soon after resolution of the immediate allergic response in both dual responding asthmatics and isolated immediate responders. It would seem that this prednisone effect is independent of its potential influence on the influx of inflammatory cells into diseased airways.

(Chest 1989; 95:314-19)

PC_{200}His = dose of histamine that doubled SRaw; EOR = early onset increase in airway reactivity.

Recently, we have reported that airway reactivity increases soon (within two hours) after resolution of the immediate response to allergen that occurs in both dual responding asthmatic subjects[1] and isolated immediate responders.[2] We have termed this phenomenon "early onset increase in airway reactivity," or EOR. Previous reports have described enhancement of airway reactivity that occurs temporally in association with the late asthmatic response.[3,4] This response is thought to require many hours to manifest clinically.

This later increase in reactivity reported by several investigative groups in dual responding asthmatic subjects has been shown to be abolished by prednisone pretreatment.[5,6] By virtue of the time at which the observations were made, it has been thought that this prednisone effect may be related to its inhibitory influence on cellular inflammatory infiltration in the airways that has been associated with the "late asthmatic response." However, cellular infiltration of airways is not thought to be associated with the immediate response in either isolated or dual phase responders to allergen.[7]

Consequently, we were interested in investigating the potential effects that prednisone pretreatment might have on EOR in both isolated immediate (henceforth called "isolated responders") and dual phase responders. We assessed histaminic reactivity before and soon after resolution of an immediate allergic response provoked in Kentucky bluegrass allergic subjects. For both the isolated and the dual phase responding subgroups, measurements were made on two separate occasions: the first occasion was done to document the occurrence and degree of EOR postallergen in each subject of the subgroup;[2] and the second was done subsequent to prednisone pretreatment (50 mg/day for three days) to ascertain its effect. To obviate the possible effects that changes in airway caliber could have on reactivity testing, we waited to reevaluate all subjects postallergen until after their immediate allergic response had resolved. It has previously been shown in hyperreactive subjects that as many as four serial, cumulative histamine challenge tests at 30 to 40 minute intervals can be done reproducibly.[8]

Methods

Subjects

Our study population consisted of two groups (Table 1). One group consisted of four subjects (one woman and three men, between 28 and 40 years of age) in whom dual phase responses after inhalation of bluegrass allergen has been previously documented. The second group consisted of four subjects (one woman and three men, between 23 and 36 years of age) who previously had been shown to manifest

*From the Departments of Internal Medicine, University of Cincinnati, Ohio, and University of Tennessee, Memphis.
This study was supported by Research grants HL-01965 and HL 34228-04 from the National Heart, Lung, and Blood Institute, grant 1599 from the Council for Tobacco Research-USA, Inc, and the Levinson Allergy Research Fund.
Presented in part at the American Thoracic Society meeting, New Orleans, May 12, 1987.
†Fellow in Allergy and Immunology.
‡Assistant Professor Medicine.
§Clinical Professor of Medicine.
||Associate Professor & Chief, Division of Pulmonary Medicine, University of Tennessee and recipient of a Research Career Development Award from the National Heart, Lung, & Blood Institute.
Manuscript received February 11; revision accepted May 3.

Table 1—*Characteristics of Subjects*

Subject No./Sex/Age	Baseline FEV_1 (Pre-/PostPrednisone)	Current Medications*
Isolated responders		
5/M/23	4.14/4.08	None
6/M/26	3.86/3.95	None
7/M/24	4.44/4.48	None
8/F/36	2.86/2.84	None
Dual Responders		
1/M/28	5.30/5.24	None
2/M/40	3.24/3.26	None
3/F/25	2.94/3.00	(Synthroid)
4/M/30	3.33/3.26	B, C Daily

*A, antihistamine; B, inhaled beta-adrenergic agonists; C, cromolyn sodium; T, slow-release theophylline compound.

only isolated immediate responses after bluegrass allergen inhalation.

All subjects had cutaneous prick test reactivity to bluegrass allergen. Intradermal cutaneous titration testing was performed using threefold increasing concentrations of bluegrass allergen in order to determine each subject's cutaneous threshold concentration to bluegrass. All studies were performed more than nine weeks after the grass pollenating season. Each subject had absent or minimal asthmatic symptoms at the time of the study. All medications that subjects had been using (Table 1) were withheld for at least 24 hours prior to allergen or histamine bronchoprovocation test days. This study was performed with the approval of the University of Cincinnati Human Research Committee and with the informed consent of the participants.

Study Design

In all subjects, airway responses were monitored by FEV_1 and SRaw. All eight subjects had baseline histamine airway challenges on two separate occasions: the first was done to document the occurrence and degree of EOR post-allergen;[2] and the second done subsequent to prednisone pretreatment (50 mg/day for three days) to ascertain its effect. On the first occasion, histamine challenges were done both 24 hours and one hour prior to bluegrass allergen bronchoprovocation. The next histamine challenge was performed after resolution of the immediate allergic response with FEV_1 had returned to within 5 percent of the preallergen value. Subsequently, hourly FEV_1 and SRaw measurements were done.

Procedures

FEV_1 measurements were made using a flow sensor; FEV_1 values reported in this study were mean values derived from three separate measurements on each occasion. The SRaw was measured using a constant volume, whole body plethysmograph, and a quiet breathing technique previously described by Krell et al[9] that we have also found useful for animal testing.[10] Briefly, airflow was measured with a heated pneumotachograph connected to a differential pressure transducer. Box pressures were also measured with a pressure transducer. Electrical outputs from both these transducers via carrier demodulators were amplified by a dual trace amplifier, and displayed simultaneously on an X-Y storage oscilloscope so that the angle described during the initial, rapid phase of inspiration could be measured and SRaw calculated from it. So that the inspiratory limb was sharply defined on the oscilloscope screen for measurement, subjects breathed slightly faster than their usual respiratory rate (20 to 24 breaths/min).

Serial dilution of histamine (64 mg to 0.03 mg/ml) were prepared at 14 day intervals from a stock supply of histamine dihydrochloride. All dilutions were made with normal saline solution. Aerosol (with a particle size of 1 to 2 μm aerodynamic mass median diameter) was generated using a nebulizer equipped with a modified dosimeter that was triggered manually upon the onset of each tidal inspiration. This dosimeter delivered a stream of compressed air to the nebulizer at 25 PSI for 0.6 s per inspiration. For histamine challenge testing, each subject inhaled ten tidal breaths of increasing histamine concentration. The concentration was doubled at six minute intervals until a 200 percent increase in SRaw was measured. The concentration of histamine chosen for initial bronchoprovocation was based on past respiratory and medication histories. All subsequent testing began with the same concentration of histamine. The provocative concentration of histamine which produced a doubling in SRaw from baseline values (the PC_{200}His) was determined by interpolation from a dose-response curve plotted on semilogarithmic paper.

Allergen skin testing and bronchoprovocation was performed using lyophilized Kentucky bluegrass extract. Allergen bronchoprovocation, using a separate nebulizer, was begun with an allergen bluegrass concentration that was one log below that of the intracutaneous threshold dose. The concentration of allergen producing at least a 3 mm wheal greater than the saline solution control was considered to be the intradermal threshold concentration. After baseline saline solution airway challenge, ten tidal breaths of threefold increasing concentrations of bluegrass allergen, were administered at ten-minute intervals until there was at least a doubling of SRaw and a 20 percent decrease in FEV_1 below the baseline values. Measurements of SRaw and FEV_1 every ten minutes thereafter were performed postallergen to detect when and how long the immediate allergic responses occurred; thereafter, hourly measurements were made to detect late asthmatic responses if they occurred.

Changes in group SRaw, FEV_1, and in the log transformed values[3] for delta PC_{200}His (log PC_{200}His on the first day before allergen minus log PC_{200}His on the subsequent occasion) were compared using Student's *t*-test. In all cases, differences were considered significant for p values less than 0.05.

Results

Characteristics of the subjects in each subgroup are shown in Table 1. The mean baseline FEV_1 of the isolated responders was similar to that for the dual phase responders (Table 1). In addition, baseline SRaw values in both subgroups prior to allergen challenge were comparable. In no subject was SRaw after the first histamine challenge (0.1 mg/ml) (Fig 1 and 2) different from baseline values.

In both subgroups of bluegrass allergic subjects, baseline PC_{200}His determinations on the two preallergen test days were similar (Fig 3). The mean baseline PC_{200}His values on day 1 for the isolated responders was higher than that determined for the dual phase responders (Fig 3).

Results of histamine challenge testing done shortly after resolution of the immediate allergic response in both subgroups are shown in Figures 1 and 2.[2] All eight subjects in both subgroups demonstrated a decrease in PC_{200}His upon resolution of the immediate response. This change was noted to occur within 80 to 140 minutes after allergen inhalation in the subjects tested. The mean decrease in PC_{200}His (Fig 3) in both subgroups (at this time compared to day 1 preallergen) was significant ($p<0.05$). Although the percentage change in reactivity status at this time was greater in the dual responders, this was not significant.

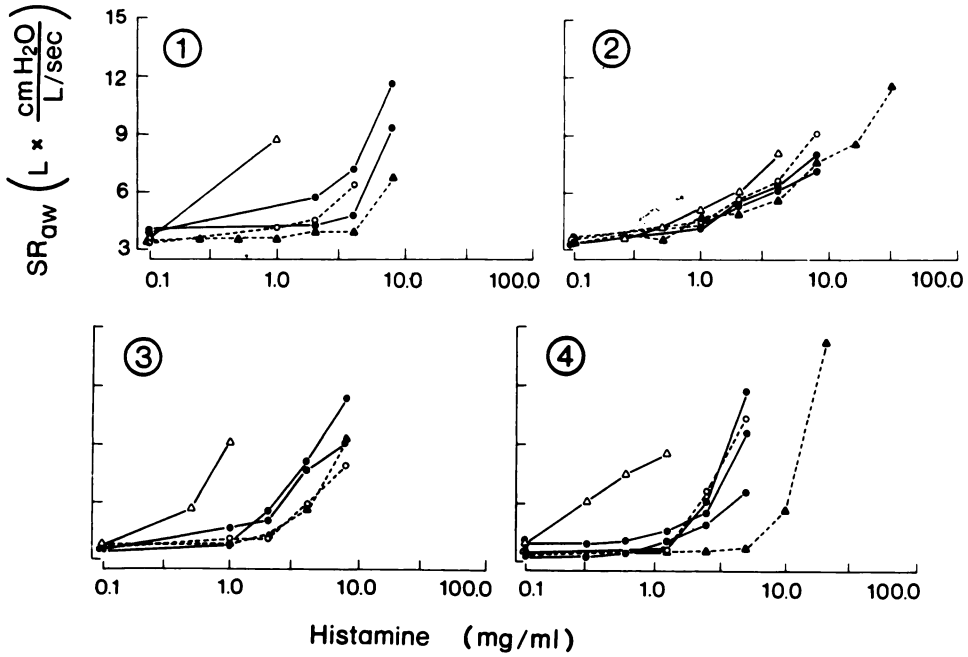

FIGURE 1. Dose-response curves demonstrating effect of inhalation of bluegrass allergen on airway reactivity to aerosolized histamine in dual responders. Curves were obtained on each of two days of prior allergen challenge *(solid circles)*; on the day of allergen exposure within ten minutes after resolution *(open triangles)* of the immediate responses;[2] and on a subsequent occasion after prednisone pretreatment, both before *(open circles, dashed lines)* and after resolution of the immediate response postallergen *(solid triangles, dashed lines)*. In each case, response was measured as SRaw before and after histamine. Each subject demonstrated a substantial increase in histamine reactivity soon after the immediate response had resolved. In contrast, after prednisone pretreatment, although baseline reactivity was unaltered by prednisone pretreatment, each subject manifested histaminic *hypo*reactivity soon after resolution of the immediate response.

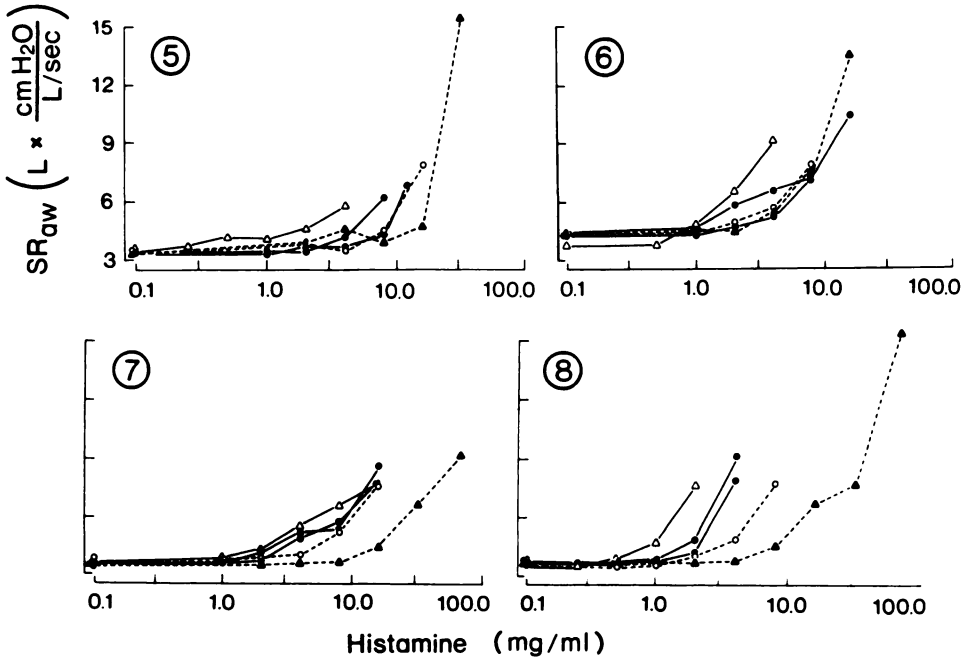

FIGURE 2. Dose-response curves demonstrating effect of inhalation of bluegrass allergen on airway reactivity to aerosolized histamine in isolated responders. Curves were obtained on each of two days prior allergen challenge *(solid circles)*: on the day of allergen exposure within ten minutes after resolution *(open triangles)* of the immediate response;[2] and on a subsequent occasion after prednisone pretreatment, both before *(open circles, dashed lines)* and after resolution of the immediate response post-allergen *(solid triangles, dashed lines)*. In each case, response was measured as SRaw before and after histamine. Each subject demonstrated a substantial increase in histamine reactivity soon after the immediate asthmatic response had resolved. In contrast, although baseline reactivity was unaltered by prednisone pretreatment, three of the four subjects manifested histaminic *hypo*reactivity soon after resolution of the immediate response.

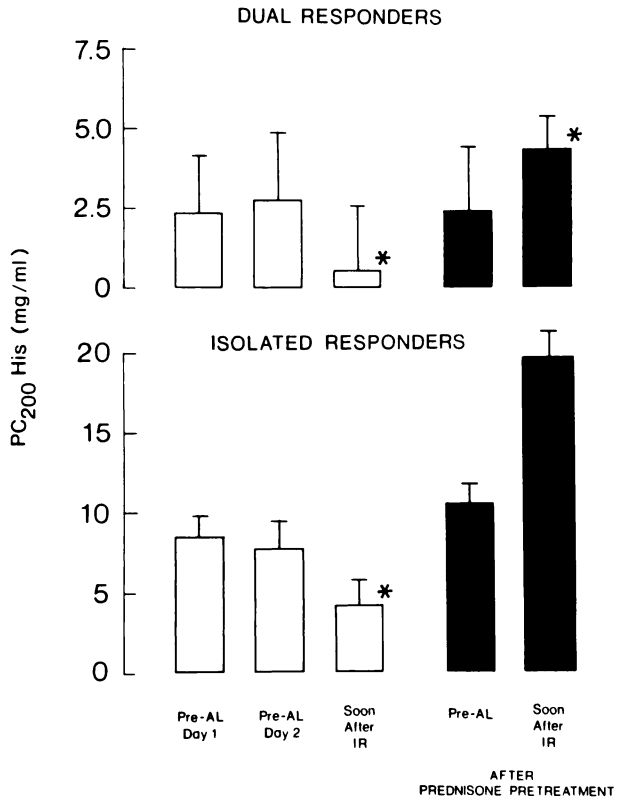

FIGURE 3. Comparison of changes in PC_{200}His after allergen to initial values (day 1) determined before allergen exposure. The PC_{200} His was derived by interpolation from dose-response curves (as specified in text). Each bar represents mean ± SE for four subjects in each subgroup. *Asterisks* indicate the values that are different from day 1 preallergen values for each subgroup ($p<0.05$). The increase in histaminic reactivity observed soon after resolution of the immediate response in both subgroups was marked. After prednisone pretreatment, substantial airway *hypo*reactivity was observed in both subject subgroups soon after resolution of the immediate allergic response and despite the fact that prednisone did not alter baseline histaminic reactivity prior to allergen exposure.

On the second occasion, subsequent to three days of prednisone pretreatment (total dose 150 mg), histamine reactivity prior to allergen was comparable in each subject to that determined on the prior occasion (Fig 1 and 2). Subsequent to prednisone pretreatment, mean PC_{200}His values were very similar to those determined on day 1 prior to prednisone pretreatment (Fig 3).

In contrast, after prednisone pretreatment, all dual phase responders and three of the four isolated responders manifested histaminic *hypo*reactivity (Fig 1 and 2) soon after resolution of the immediate response (in relation to their reactivity status on day 1 preallergen). This occurred despite the fact that prednisone did not appear to affect the degree of the immediate response elicited by allergen in any of the subjects tested. The change in reactivity status soon after resolution of the immediate response was clearly significant ($p<0.05$) in the subgroup of dual responders (Fig 3). Although the decrease in histaminic reactivity in the isolated responders was also substantial, this was not significant for the subgroup ($p<0.09$) in relation to day 1 preallergen values. However, in comparison to the PC_{200}His values determined soon after the immediate responses prior to prednisone pretreatment (rather than day 1 values), this decrease in reactivity for the subgroup was highly significant ($p<0.01$).

Discussion

In this study, we have demonstrated that the increase in airway reactivity that occurs soon after resolution of the immediate response induced by allergen (*ie*, EOR) is inhibited by prednisone. It has been our experience that this EOR occurs in both isolated[2] and in dual phase responders to allergen.[1] Prednisone pretreatment abolished this effect in both subject groups. However, in neither of these groups did prednisone have an influence on baseline airway reactivity prior to allergen challenge. Soon after resolution of the immediate response in both atopic groups, at a time when their allergen-induced airway constriction had resolved, we were surprised to find that their histaminic reactivity had actually *decreased* significantly in relation to values determined prior to allergen exposure. To our knowledge, this is the first report of such an effect of prednisone pretreatment.

This profound histaminic hyporeactivity in prednisone pretreated subjects occurring soon after resolution of the immediate allergic response is a curious phenomenon. We are unaware of such an observation or an analogous one having been made before. That prednisone inhibited EOR in both isolated and dual phase responders suggests to us that its effect may be independent of its potential influence on the influx of inflammatory cells into diseased airways. In this and our previous experience,[1,2] we have determined that EOR occurs within 140 minutes after allergen exposure. A recent publication concerning the percentages of inflammatory cells present in bronchoalveolar lavage fluid obtained at two to three hours after allergen challenge in dual responders, and at six to seven hours in isolated responders showed no significant changes between these groups and compared to control subjects.[7] Therefore, the fact that EOR postallergen occurs in both isolated and dual phase responders suggests to us that this increase in reactivity is not reflected by percentage changes in the neutrophilic, eosinophilic, or lymphocytic composition of lavage fluids from such atopic subjects. It is conceivable, however, that such a change in reactivity in both isolated and dual phase responders may be a result of inflammatory cell accumulation in the airway tissue microvasculature. Such cellular accumulation may not be expressed on the airway surface, and hence, in lavage fluids. Whether or not there are other changes in bronchoal-

veolar lavage fluids which do relate to mechanisms that are responsible for EOR remains to be fully investigated. It seems possible that there may be changes in the content of bronchoactive mediators in lavage fluids from such allergic subjects if these substances are elaborated by airway cells and diffuse into airway lumina after allergen exposure, as has been shown in nasal washings from subjects with allergen-induced rhinitis.[11]

In both isolated and dual phase responders, it appears that the immediate response is at least partially related to the release of histamine.[12] However, this histamine release is presumably not responsible for EOR in that serial bronchial challenges with histamine have not been shown to increase reactivity after histamine-induced airway constriction resolves.[8] Thus, other factors are presumably responsible for the immediate allergic response.

Taken with our previous experience,[1,2] the present study suggests to us that EOR postallergen may be common to both isolated and dual phase responders. This increase in reactivity may result from the release of mediators during or soon after an allergen-induced immediate response that potentiates airway smooth muscle tone to histamine at that time. In this regard, the recent report by Arm et al[13] is of substantial interest. In this study, baseline measurements of specific airway conductance (SGaw) were made in five asthmatic subjects before and after inhalation of a dose of leukotriene E_4 which caused a 35 percent fall in SGaw that remitted spontaneously to baseline values over the ensuing 60 to 90 minutes. When SGaw had returned to baseline values, these investigators found that all five subjects manifested a mean increase in airway histamine reactivity of 3.5 fold.[13]

Although it is unclear at present what specific mediators are responsible for the immediate and late phase response in man, studies *in vitro* of human airways have provided some insights. Allergenic challenge *in vitro* of sensitized human bronchi produces airway muscle contraction that appears to have both an "early" and "late phase," the latter occurring within 20 minutes of exposure.[14] Such stimulation of sensitized human bronchi has also been shown to result in the formation of a number of cyclooxygenase metabolites of arachidonic acid.[15,16] In addition, it appears that allergen-induced bronchospasm *in vitro*[17] involves histamine and slow-reacting substance of anaphylaxis (now known to consist of leukotrienes C_4, D_4 and E_4). These mediators are potent airway spasmogens and may also augment responsiveness to other bronchoconstrictors both *in vitro*[18] and *in vivo*,[19] as may "platelet activating factor."[20]

Linked with these observations, our study leads us to speculate that prednisone may reduce histaminic responsiveness after the immediate allergic response by affecting the production of mediators that would otherwise result. Repeated allergen challenges *in vitro* of sensitized airway preparations leads to rapid histaminic tachyphylaxis.[21-23] Perhaps, such histaminic hyporeactivity might also develop *in vivo* if allergen exposure of asthmatic subjects did not also lead to the generation of other bronchoconstricting mediator(s).[24] Thus, these mediators might, in fact, mask histaminic hyporeactivity that may develop *in vivo* postallergen. By inhibiting the generation of these mediators, prednisone pretreatment may reveal this hyporeactivity. Alternatively, it is possible that prednisone pretreatment may stimulate the production of other factors which (over)compensate for the airway constriction induced by allergen (and histamine) exposure. Such mediators may affect airway muscle tone and reactivity status without necessarily affecting baseline airway caliber as it is indirectly reflected by SRaw or FEV_1. Future studies to investigate these issues are clearly warranted.

ACKNOWLEDGMENTS: The authors thank Mary Kay Winton and Anita Farrell for their invaluable help in preparing the manuscript.

References

1 Thorpe JE, Steinberg D, Bernstein IL, Murlas CG. Bronchial reactivity increases soon after the immediate response in dual-responding asthmatic subjects. Chest 1987; 91:21-25

2 Steinberg DR, Bernstein DI, Bernstein IL, Murlas CG. Bronchial reactivity increases early in both immediate and dual phase responders to allergen. J Allerg Clin Immunol 1987; 79:249

3 Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen induced increase in non-allergic bronchial reactivity. Clin Allergy 1977; 7:503-13

4 Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allerg Clin Immunol 1982; 70:170-77

5 Booij-Noord H, Orie NGM, de Vries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allerg Clin Immunol 1971; 48:344-54

6 Pepys J, Hutchcroft BJ. Bronchial provocation test in etiologic diagnosis and analysis of asthma. Am Rev Respir Dis 1975; 112:829-59

7 De Monchy JGR, Kauffman HF, Vange P, Koeter GH, Jansen HM, Sheiter HJ, et al. Bronchoalveolar eosinophillia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373-76

8 Ruffin RE, Alpers JH, Crockett AJ, Hamilton R. Repeated histamine inhalation tests in asthmatic patients. J Allerg Clin Immunol 1981; 67:285-89

9 Krell WS, Agrawal KP, Hyatt RE. Quiet-breathing vs panting methods for determination of specific airway conductance. J Appl Physiol 1984; 57:1917-22

10 Roum JH, Murlas C. Ozone-induced changes in muscarinic bronchial reactivity by different testing methods. J Appl Physiol 1984; 57:1783-89

11 Creticos PS, Peters SP, Adkinson NR Jr, Naclerio RM, Hayes EC, Norman PS, et al. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. N Engl J Med 1984; 310:1626-30

12 Rocklin RE, Findlay SR. Immunologic mechanisms and recent advances in asthma. In: Weiss EB, Segal MS, and Stein M, eds. Bronchial asthma: mechanisms and therapeutics. Boston: Little, Brown, 1985; 41-51
13 Arm JP, Spur BW, Lee TH. Leukotriene E4 enhances airway histamine responsiveness in asthmatic subjects. Thorax 1987; 42:220
14 Adams GK, Lichtenstein LM. Indomethacin enhances response of human bronchus to antigen. Am Rev Respir Dis 1985; 131:8-10
15 Schulman ES, Adkinson NF, Adams GK, Lichtenstein LM, Newball HH. Anaphylactic release of thromboxane A2 from human bronchi and parenchyma. J Allerg Clin Immunol 1980; 65:235
16 Schulman ES, Adkinson NF Jr, Newball HH. Cyclo-oxygenase metabolites in human lung anaphylaxis: airway vs parenchyma. J Appl Physiol 1982; 53:589-95
17 Adams GK, Lichtenstein LM. In vitro studies of antigen-induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. J Immunol 1979; 122:555-62
18 Lee TH, Austen KF, Corey EJ, Drazen JM. Leukotriene E4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. Proc Natl Acad Sci USA 1984; 81:4922-25
19 Stewart AG, Thompson DC, Fennessy MR. Leukotriene D4 potentiates histamine-induced bronchoconstriction in guinea pigs. Agents Actions 1984; 15:146-52
20 Benveniste J. A role for paf-acether (platelet activating factor) in bronchial hyperreactivity? Am Rev Respir Dis 1987; 135:2-4
21 Anderson WH, Krzanowski JJ, Polson JB. Characteristics of histamine tachyphylaxis in canine tracheal smooth muscle. Arch Pharmacol 1979; 308:117-25
22 Brink C, Duncan PG, Douglas JS. Histamine tachyphylaxis in canine isolated airways: role of endogenous prostaglandins. J Pharm Pharmacol 1982; 34:199-201
23 Krzanowski JJ, Anderson WH, Polson JB. Prostaglandin mediated histamine tachyphylaxis in subhuman primate tracheal smooth muscle. Arch Int Pharmacodyn 1980; 247:155-62
24 Murray JJ, Tonnel AB, Brash AR, Roberts LJ, Gosset P, Workman R, et al. Release of prostaglandin D2 into human airways during acute antigen challenge. N Engl J Med 1986; 315:800-804