

Effect of inhaled corticosteroids on peripheral blood eosinophil counts and density profiles in asthma

Patricia M. Evans, BSc, Brian J. O'Connor, MRCPI,
Richard W. Fuller, MRCP (UK), Peter J. Barnes, DSc, FRCP, and
K. Fan Chung, MD, FRCP London, United Kingdom

Background: Inhaled corticosteroids are effective in the treatment of asthma and reduce bronchial hyperresponsiveness. We examined the effect of inhaled budesonide (1600 µg/day for 14 days) on the number and density profile of peripheral blood eosinophils in 10 patients with mild stable asthma in a double-blind, placebo-controlled, crossover trial.

Methods: At the end of each study period, blood was collected immediately before measurement of bronchial responsiveness calculated as the concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second (PC_{20}). Plasma leukocyte suspension obtained after sedimentation of blood with 46% dextran was layered on a Percoll continuous-density gradient. After centrifugation, 20 sequential aliquots of increasing density gradients were removed for differential counts. Eosinophils recovered from density of gradients less than 1.080 gm/ml were defined as hypodense.

Results: Budesonide treatment resulted in a fall in mean eosinophil count from $0.37 \pm 0.05 \times 10^9 L^{-1}$ after placebo to $0.16 \pm 0.03 \times 10^9 L^{-1}$ ($p < 0.01$). A significant reduction occurred in the hypodense eosinophil population from $51.6\% \pm 4.3\%$ after placebo to $38.1\% \pm 3.1\%$ after budesonide ($p < 0.05$). Mean log PC_{20} increased from -0.26 ± 0.12 after placebo to -0.004 ± 0.14 after budesonide ($p < 0.01$).

Conclusion: We conclude that inhaled steroid therapy reduces the number of circulating hypodense eosinophils, which is an effect that may underlie the improvement in bronchial hyperresponsiveness. (*J ALLERGY CLIN IMMUNOL* 1993;91:643-50.)

Key words: Corticosteroids, eosinophils, eosinophil density, budesonide, bronchial hyperresponsiveness

Inhaled corticosteroid therapy remains one of the most effective treatments for chronic asthma and reduces bronchial hyperresponsiveness.¹⁻⁴ Inhaled steroids have also been shown to inhibit the submucosal inflammation present in asthmatic airways, with a reduction in the number of eosinophils and mast cells.⁵ Eosinophils may play an important role in asthma,

Abbreviations used

FEV ₁ :	Forced expiratory volume in 1 second
PC ₂₀ :	Provocative concentration of methacholine causing a 20% fall in FEV ₁
PIPES:	Piperazine- <i>N,N'</i> -bis(2-ethanesulfonic acid)
HBSS:	Hank's balanced salt solution
RI:	Refractive index
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL-3, IL-5:	Interleukin-3, interleukin-5

From the Department of Thoracic Medicine, National Heart and Lung Institute, Royal Brompton National Heart and Lung Hospital, London.

Supported by the Medical Research Council (UK).

Received for publication Jan. 28, 1992.

Revised Sept. 30, 1992.

Accepted for publication Oct. 2, 1992.

Reprint requests: K. F. Chung, Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse St., London SW3 6LY, United Kingdom.

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/43173

and it has been postulated that eosinophils may release granule protein contents such as major basic protein and eosinophil cationic protein to cause cytotoxic damage to the airway epithelium in asthma.⁶ Recently eosinophils isolated from venous blood of patients

TABLE I. Anthropometric details, baseline FEV₁, airway responsiveness to methacholine, and total peripheral blood eosinophil count

Subject no.	Gender	Age	FEV ₁ (% predicted)	Medication	Methacholine PC ₂₀ (mg/ml)	Total eosinophil count ($\times 10^9/L$)
1	M	21	98	β_2	0.51	0.62
2	F	20	90	β_2	0.35	0.45
3	F	20	100	β_2	0.13	0.46
4	F	20	93	β_2	0.40	0.40
5	M	34	80	β_2	0.30	0.29
6	M	36	108	β_2	2.23	0.14
7	M	22	92	β_2	1.05	0.31
8	M	33	113	β_2	2.57	0.22
9	M	46	93	β_2	0.48	0.53
10	F	22	95	β_2	0.33	0.57

β_2 , Inhaled β_2 -adrenergic agonists.

with asthma have been shown to consist of a more heterogeneous population in terms of their density profiles. Thus up to 40% of peripheral eosinophils were found to be hypodense (density less than 1.080 mg/ml).⁷ It has been postulated that these eosinophils represent immature or "activated" forms or a mixture of both types.⁸ Inhaled corticosteroid therapy inhibits the number of circulating venous eosinophils in asthma,³ and in vitro studies have shown that steroids inhibit the induction of human hypodense eosinophils.⁹ In the present study we have examined whether inhaled corticosteroid therapy has any effect on the density profile of peripheral blood eosinophils obtained from asthmatic patients. In addition, we have examined the relationship between the changes in hypodense eosinophils and the improvement in bronchial responsiveness induced by inhaled steroid therapy.

MATERIAL AND METHODS

Subjects

Ten adult subjects with mild asthma who were non-smokers (6 men; age range, 20 to 46 years) gave informed written consent to participate in the study which was approved by the Ethics Committee of the Royal Brompton National Heart and Lung Hospital (Table I). The study took place between the months of December and May 1989. All subjects were atopic according to positive results of skin prick tests to a range of common environmental allergens such as house dust mite and mixed grass pollen. Subjects had occasional symptoms that were controlled only by the use of inhaled β_2 -adrenoceptor agonists when necessary. Baseline forced expiratory volume in one second (FEV₁) was greater than 80% predicted. All subjects had a peripheral eosinophil count of greater than $10^8 L^{-1}$. At the time of entry to the study, asthmatic subjects had been stable over the previous 6 weeks.

The study was double-blind, cross-over, and placebo-controlled. Five subjects were randomly allocated to receive inhaled budesonide (800 μg , twice a day; i.e., 1600 μg per

day) during the first treatment period of 14 days. The remaining five subjects received a matched placebo inhaler during the first treatment period. Budesonide and matched placebo were inhaled with use of a multidose dry powder delivery system (Turbohaler; Draco AB, Division of Astra Pharmaceutical Production, Lund, Sweden). After a wash-out period of 4 weeks, the treatments were reversed for the second 14-day treatment period. On the morning of the last day of each treatment period, 50 ml of venous blood was obtained, and airway responsiveness to methacholine was determined. Subjects were requested to refrain from using their β_2 -agonist inhalers or drinking caffeinated beverages for at least 8 hours before arriving in the laboratory.

Quantification of white blood cell numbers

Total white blood cell and differential count were performed on whole blood obtained for density analysis, and the fractions isolated from Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradients with a Technicon H.1 cell counter (Technicon Chemicals Company, Tournai, Belgium). The cell counter defines different cell types by means of a peroxidase method of staining.¹⁰ To establish the accuracy of the Technicon peroxidase method, initial samples were also quantitated with Kimura stain with a Neubauer counting chamber.¹¹ The coefficient of variation of the Technicon H.1 system was less than 10%.

Preparation of Percoll gradients

A 1 L stock solution of Percoll sufficient for the whole study, with a density of 1.084 gm/ml, was prepared with piperazine-*N,N'*-bis(2-ethanesulfonic acid PIPES) buffer for the dilution of Percoll to maintain constant pH and osmolarity according to the method of Fukuda et al.⁷ In brief, Percoll (500 ml; density, 1.130) was mixed with 45 ml $\times 10$ PIPES buffer and 12 ml of distilled water to form the heavy Percoll solution. One part of the heavy Percoll solution was mixed with nine parts of $\times 1$ PIPES buffer to form the light solution. A Percoll solution was made to a density of 1.084 gm/ml, as determined by a pycnometer (Arthur H. Thomas Co., Philadelphia, Pa.) by mixing the heavy and light solutions. The solution was sterilized by passage through a

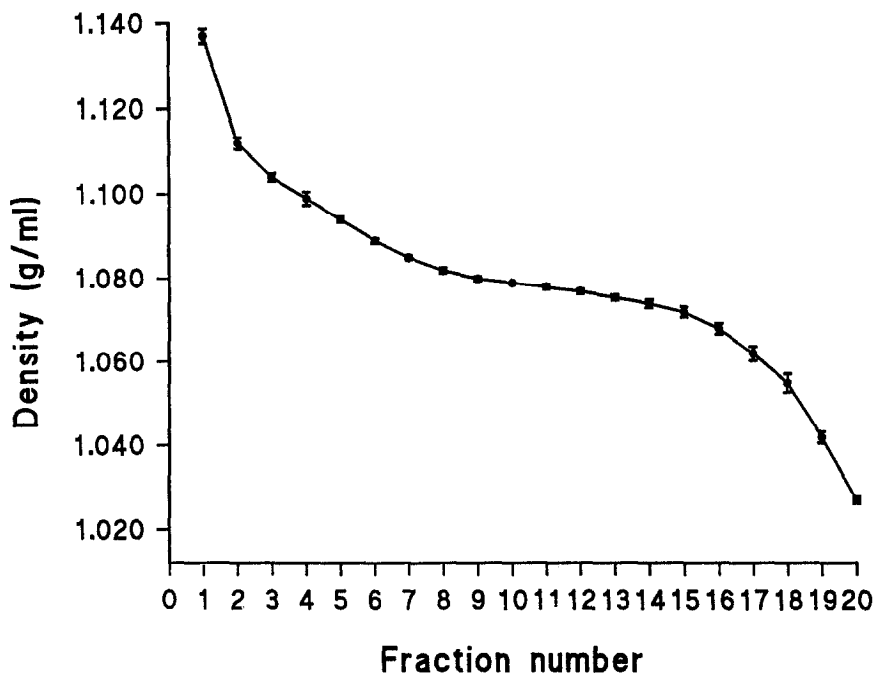


FIG. 1. Density profile measured by a refractometer of 20 continuous fractions of a Percoll gradient prepared by centrifugation at 20,000 *g* for 15 minutes at 22° C. Gradient was used to determine density profile of eosinophils. Each point represents mean $\pm$ SEM of four determinations.

0.2 μ m Millipore filter (Minisart N; Sartorius, Epsom, Surrey, U.K.) and stored at 4° C. Continuous gradients were prepared by centrifuging 10 ml of the 1.084 gm/ml Percoll solution in a polycarbonate tube at 20,000 *g* for 15 minutes at 22° C. Four separate gradients per subject were prepared for each density profile analysis.

Studies in our laboratory of healthy subjects demonstrated that peripheral blood eosinophils had densities that were predominantly in the range of 1.081 to 1.095 gm/ml, which is similar to that reported previously by other groups.^{7, 12} Therefore we defined eosinophils with density of less than 1.080 as being hypodense. To account for the variability in eosinophil number between patients and the effect of drug treatment, the number of hypodense eosinophils was expressed as a percentage of total eosinophils recovered on the Percoll gradients. In two asthmatic subjects, we found the density profile of peripheral blood eosinophils to be reproducible within a 2-week period on two repeated determinations.

Fractionation of granulocytes

Venous blood (50 ml) was collected into 10 ml acid citrate dextrose anticoagulant solution. Leukocytes were obtained by sedimenting 5 volumes of blood with 1 volume of 6% dextran 110 for 45 minutes at 22° C. The leukocyte-rich plasma was collected, centrifuged at 500 *g* for 15 minutes at 4° C, and resuspended to 4 ml. A 1 ml aliquot of the cell suspension was layered carefully onto each gradient and centrifuged (1600 *g* for 20 minutes at 22° C) in a swing-rotor. After centrifugation 20 sequential 0.5 ml fractions

containing dispersed granulocytes were collected from the bottom of the tube with a peristaltic pump connected to a microfractionator (FRAC-100; Pharmacia, Uppsala, Sweden). One hundred microliter samples were taken from each fraction, and the density of the fraction was determined by measurement of its refractive index (RI) by means of a refractometer. The remaining 1.9 ml samples were diluted fivefold in Hank's balanced salt solution (HBSS), centrifuged at 200 *g* for 15 minutes at 4° C, and resuspended in 200 μ l HBSS for differential white blood cell counts with the Technicon H.1 counter.

Calibration of density gradients

The relationship between the RI and the density of Percoll was established to enable the density of fractions obtained from the gradients to be determined by measurement of their RI as previously described.⁷ The density of eight different Percoll solutions was calculated with a 10 ml pycnometer, and RI of each solution was measured at 22° C with a refractometer (ABBE 60; Bellingham and Stanley Ltd., Tunbridge Wells, Kent). Density and RI demonstrated a positive linear relationship as described by the following equation: density = (RI - 1.182)/0.153 ($r = 0.995$). Each continuous gradient produced 20 fractions ranging in density from 1.027 to 1.137 gm/ml as shown in Fig. 1.

Measurement of methacholine responsiveness

Methacholine inhalation tests were performed by means of a standardized method. After measurement of baseline

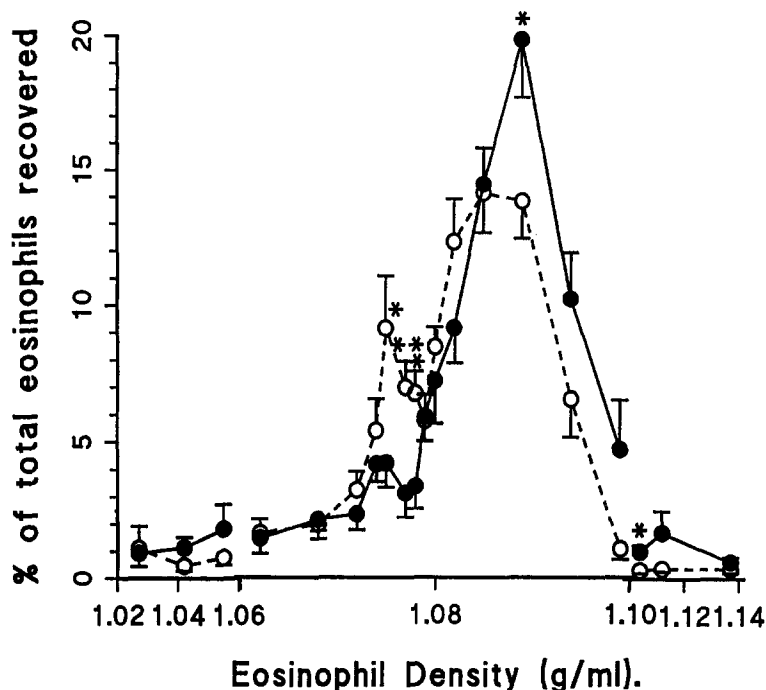


FIG. 2. Density profiles of eosinophils recovered from venous blood of 10 asthmatic subjects after placebo treatment (----) and after treatment with inhaled budesonide (—). Eosinophil counts are expressed as a percentage of total eosinophils recovered from Percoll gradients. Each point represents a measurement at that particular density fraction. Note that the scale for eosinophil density between 1.06 and 1.10 gm/ml has been expanded to allow for clarity of presentation of results. * indicate $p < 0.05$ for comparison between placebo and steroid treatment periods. Data shown as mean $\pm$ SEM.

FEV₁ with a dry wedge spirometer (Vitalograph, Buckingham, U.K.), each subject inhaled five breaths of 0.9% saline solution followed by five breaths of doubling concentrations (0.125 to 32 mg ml⁻¹) of methacholine dissolved in 0.9% saline solution delivered at 3-minute intervals. The aerosols were generated with a nebulizer attached to a breath-activated dosimeter (MEFAR, Brescia, Italy). The output of the nebulizer was 9 mm³ per activated breath, with particle size of mean mass diameter of 3.5 μ m. The response of the FEV₁ to 0.9% sodium chloride and to increasing concentrations of methacholine was measured at 1 minute after each inhalation. A log-dose response was constructed, and the provocative concentration causing a 20% fall in FEV₁ (log PC₂₀) was obtained by linear interpolation.

Reagents

The following drugs and chemicals were used: acetyl- β -methylcholine chloride (methacholine) (Sigma, Poole, Dorset, U.K.), Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), and HBSS (Gibco, Paisley, U.K.). Kimura stain was prepared with reagents purchased from Sigma, U.K.⁸ The composition of the PIPES buffer was 25 mmol/L piperazine-*N,N'*-bis(2-ethanesulfonic acid), 110 mmol/L NaCl, 5 mmol/L KCl, 40 mmol/L NaOH, and 5.4 mmol/L glucose (pH 7.4) (Sigma, U.K.). The composition of the acid

citrate dextrose was 85 mmol/L sodium citrate, 71 mmol/L citric acid, and 111 mmol/L glucose (Sigma, U.K.).

Data analysis

Data are reported as mean $\pm$ SEM. Differences in number of white blood cells or in the log PC₂₀ to methacholine were analyzed with use of Student's *t* test for paired samples. Differences in granulocyte density expressed as a mean density or percent of cells in the hypodense range were compared with use of Mann-Whitney U-test. The change in number or percentage of hypodense eosinophils was assessed with use of the Wilcoxon signed rank-sum test. All correlations were assessed with use of Spearman regression analysis. To exclude the possibility of carryover effect in our crossover design, we tested for treatment-period interaction.¹² All analyses were two-tailed, and the null hypothesis was rejected at $p < 0.05$.

RESULTS

Peripheral cell counts

No significant difference occurred in the peripheral white cell count between placebo and budesonide periods. Neutrophil, lymphocyte, and monocyte counts were also not significantly different. However, mean

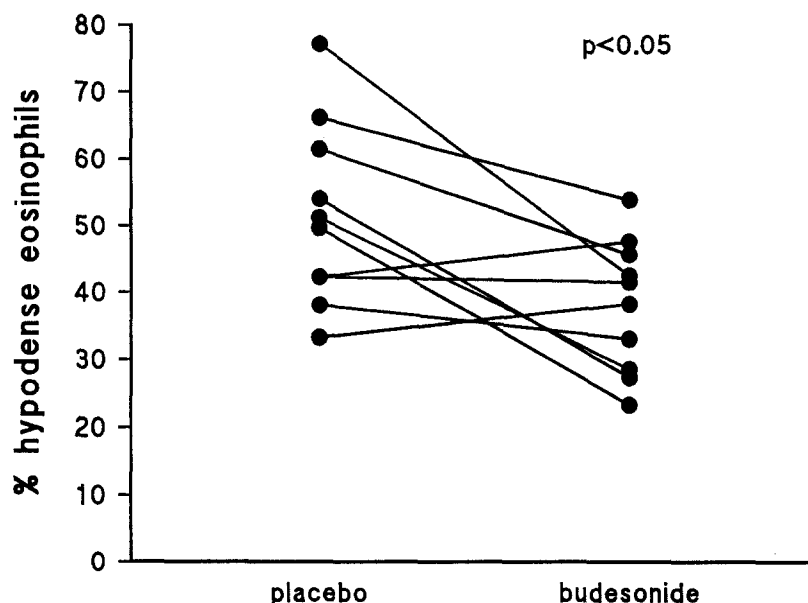


FIG. 3. Hypodense eosinophils (with density <1.080 as defined on buoyant density gradients of Percoll) expressed as a percentage of total eosinophils recovered from Percoll gradients in 10 asthmatic subjects after placebo treatment and after treatment with inhaled budesonide. There was a significant reduced number of hypodense eosinophils after steroid treatment ($p < 0.05$).

eosinophil count was lower during budesonide treatment period ($0.16 \pm 0.03 \times 10^9 \text{ L}^{-1}$) compared with placebo treatment period (0.37 ± 0.05 ; $p < 0.01$).

Eosinophil density gradients

Recovery of eosinophils (expressed as a percentage of the total eosinophil count) was similar in the placebo ($50.7\% \pm 0.9\%$) and in the budesonide ($50.3\% \pm 0.4\%$) treatment periods. The density distribution of the eosinophils during the two periods are shown in Fig. 2. During the placebo period two peaks were observed at 1.089 and 1.075 gm/ml. By contrast, after budesonide treatment, only one major peak at a density of 1.089 was found, with fewer cells present in the fractions of lower density. The effect of budesonide compared with placebo on the percentage of hypodense eosinophils (i.e., densities <1.080) is shown in Fig. 3. Mean percent hypodense eosinophils fell from $51.6\% \pm 4.3\%$ on placebo to $38.2\% \pm 3.1\%$ while on steroid therapy ($p < 0.05$). Six subjects who showed the greatest percentage of hypodense eosinophils (above 45%) while on placebo treatment demonstrated the greatest fall while on budesonide treatment (from 12% to 35% of total eosinophils). A significant correlation occurred between the percent hypodense eosinophil on placebo treatment and the reduction in hypodense eosinophils after bu-

desonide ($r = 0.75$; $p < 0.05$). There was no significant treatment-period interaction.

Bronchial responsiveness to methacholine

There was a significant improvement in log PC₂₀ to methacholine while on budesonide treatment (placebo, -0.26 ± 0.12 ; budesonide, 0.004 ± 0.14 ; $p < 0.01$; Fig. 4).

Relationship between eosinophil parameters and methacholine responsiveness

A significant correlation occurred between log PC₂₀ and the peripheral blood eosinophil count ($r = -0.66$; $p < 0.05$); however, there was no correlation with the absolute number of hypodense eosinophils ($r = 0.29$), or the percentage hypodense eosinophils ($r = -0.07$). The change in log PC₂₀ between placebo and budesonide treatments did not correlate with the reduction in number of hypodense eosinophils ($r = 0.32$).

DISCUSSION

In our study we found a mean 52% of circulating eosinophils (range, 32% to 78%) to be within the hypodense range, that is, with a density <1.080 as defined on buoyant density gradients of Percoll in patients with mild asthma. This range of hypodense eosinophils is in close agreement with those reported

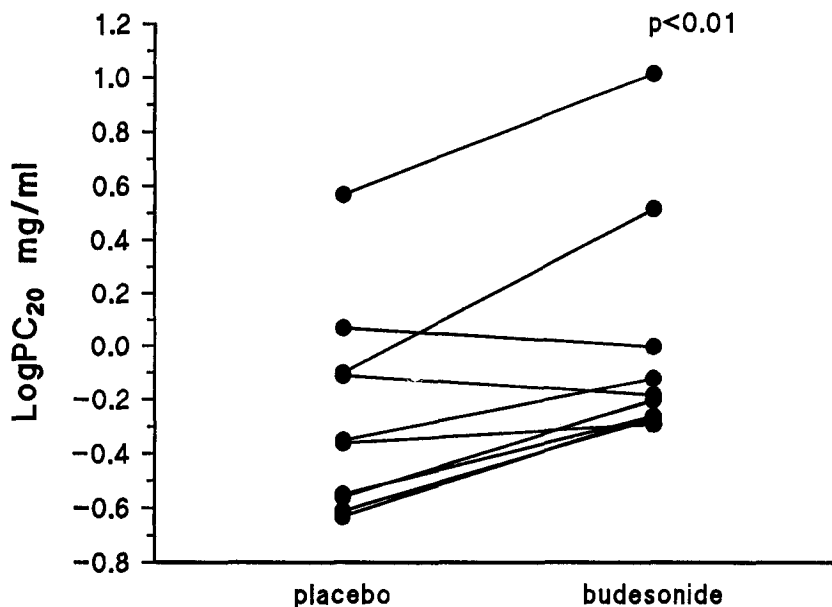


FIG. 4. Airway responsiveness, expressed as log PC₂₀, in 10 asthmatic subjects after placebo treatment and after treatment with budesonide. There was a significant increase in log PC₂₀ after budesonide treatment ($p < 0.01$).

previously by Fukuda et al.⁷ and Shult et al.¹³ Inhaled corticosteroids administered over 14 days, significantly reduced the total number of circulating eosinophils and the percentage of eosinophils that were hypodense, changes that were associated with an improvement in bronchial responsiveness to methacholine. We found no significant correlation between the decrease in PC₂₀ and the fall in total or in hypodense eosinophil numbers. Overall, there was a 1.8-fold improvement in airway responsiveness associated with a nearly threefold reduction in the number of circulating hypodense eosinophils after inhaled steroid therapy.

Although the mechanisms by which hypodense eosinophils are generated in asthma have not been precisely defined, current evidence suggests that these may develop as a result of exposure to cytokines. Culture of normodense eosinophils with human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), or interleukin-5 (IL-5) in the presence of fibroblasts results in production of hypodense cells.¹⁴⁻¹⁶ Transcripts of messenger RNA for cytokines such as IL-5 and GM-CSF can be detected by in situ hybridization in biopsies of airways of patients with symptomatic asthma,^{17, 18} suggesting that these cytokines may activate tissue eosinophils locally and prolong their survival. Sera obtained from patients with asthma are also capable of increasing the survival of eosinophils, probably because of the presence of IL-5 and GM-CSF.¹⁹

Corticosteroids have been shown to inhibit the prolonged survival and to prevent the induction of hypodense eosinophils in culture induced by GM-CSF.⁹ They also have the capacity of inhibiting eosinophil colony formation in peripheral blood and bone marrow cultures.²⁰ In addition, they can inhibit the generation of various cytokines such as IL-3, IL-5, and GM-CSF from lymphocytes.^{21, 22} These observations suggest that there may be different sites of action of corticosteroids underlying the reduction in the number of hypodense eosinophils. In the present study inhaled corticosteroids may be acting locally in the airways to inhibit cytokine production and release. After inhalation of 500 µg budesonide, blood levels of approximately 2 nmol/L are achieved,²³ a concentration that would be ineffective in inhibiting any of the known effects of corticosteroids discussed above. Toogood et al.²⁴ have shown that inhaled budesonide is effective in controlling asthma symptoms, whereas oral budesonide taken at a dose to achieve blood levels similar to those achieved with inhaled budesonide was not. This observation supports the hypothesis that part of the effect of inhaled steroids is mediated locally within the lung. Inhibition of local release of cytokines such as IL-5 and GM-CSF may be an important topical effect of inhaled corticosteroids, which may underlie the reduction in circulating hypodense eosinophils. Inhaled steroids have been shown to reduce eosinophil numbers in biopsies of the airway mucosa of asthmatic patients and to reduce the levels of eosinophilic cat-

ionic protein, a specific product of activated eosinophils, in bronchoalveolar lavage fluid.²⁵ It is also possible that a decrease in survival time of eosinophils in the circulation may account for the decrease in total numbers of circulating eosinophils.

Eosinophils have been implicated in the pathogenesis of bronchial hyperresponsiveness in asthma.²⁶ Hypodense eosinophils obtained from patients with asthma demonstrate an augmented capacity to release mediators such as leukotriene C₄.²⁷ Activated eosinophils as evidenced by positive staining of eosinophil cationic protein, have been shown to be present within the submucosa of asthmatic airways, particularly just beneath the subepithelial collagen layer and within the epithelium.^{28, 29} Inhibition of eosinophil activation as may be reflected by a reduction in the number of hypodense eosinophils as shown in our study may be related to the improvement in bronchial responsiveness after inhaled corticosteroid therapy. Other investigators have shown that the number of circulating hypodense eosinophils increases during the late phase response to allergen in patients with allergic asthma.³⁰ More direct evidence for the contribution of eosinophils to bronchial hyperresponsiveness in asthma may be obtained by studying airway tissue eosinophils after steroid therapy.

We thank Dr. J. Burman, Department of Pathology, for allowing us to use the Technicon Cell Counter in his department and Draco (Sweden) for their support during the course of this study.

REFERENCES

1. Chung KF, Clark TJH. Corticosteroids. In: Clark TJH, Godfrey S, Lee TH, eds. *Asthma*, third ed. London: Chapman Hall Medical, 1992:416-48.
2. Kraan J, Koeter GH, Van der Mark TW, Sluiter HJ, De Vries K. Changes in bronchial hyperactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J ALLERGY CLIN IMMUNOL* 1985;76:628-36.
3. Kerrebijn KF, Von Essen-Zandvliet EEM, Neijens HJ. Effect of long-term treatment with inhaled corticosteroids and beta-agonists on bronchial responsiveness in asthmatic children. *J ALLERGY CLIN IMMUNOL* 1987;79:653-9.
4. Juniper EF, Kline PA, Vanzileghen MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in steroid-dependent asthmatics. *Am Rev Respir Dis* 1990;142:832-6.
5. Laitinen LA, Laitinen A, Haahtela T. Treatment of eosinophilic airway inflammation with inhaled corticosteroid, budesonide, in newly diagnosed asthmatic subjects. *Eur Respir J* 1991;4:342.
6. Gleich GJ, Flavahan NA, Fujisaura T, Vanhoute PM. The eosinophil as a mediator of damage of respiratory epithelium: a model for bronchial hyperactivity. *J ALLERGY CLIN IMMUNOL* 1988;81:776-81.
7. Fukuda T, Dunnette SL, Reed CE, Ackerman SJ, Peters MS, Gleich GJ. Increased numbers of hypodense eosinophils in the blood of patients with bronchial asthma. *Am Rev Respir Dis* 1985;132:981-5.
8. Fukuda T, Gleich GJ. Heterogeneity of human eosinophils. *J ALLERGY CLIN IMMUNOL* 1989;83:369-73.
9. Lamas AM, Leon OG, Schleimer RP. Glucocorticoids inhibit eosinophil responses to granulocyte macrophage colony-stimulating factor. *J Immunol* 1991;147:254-9.
10. Saunders AM. Hemalog D system—recent development. *Advances in Automated Analysis*, Technicon International Congress. Tarrytown, New York, Mediad Inc, 1972;3:27-32.
11. Kimura I, Moritani Y, Tanizaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. *Clin Allergy* 1973;3:195-202.
12. Altman DG. *Practical statistics for medical research*. 1st ed. London: Chapman and Hall, 1991:466-71.
13. Shult PA, Lega M, Jadidi S, et al. The presence of hypodense eosinophils and diminished chemiluminescence response in asthma. *J ALLERGY CLIN IMMUNOL* 1988;81:429-37.
14. Rothenberg ME, Owen WF Jr, Siberstein DS. Human eosinophils have prolonged survival, enhanced functional properties and become hypodense when exposed to human interleukin-3. *J Clin Invest* 1988;81:1986-92.
15. Rothenberg ME, Petersen J, Stevens RL. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity and sustained antibody-dependent cytotoxicity. *J Immunol* 1989;143:2311-6.
16. Owen WF, Rothenberg ME, Silberstein DS, et al. Regulation of human eosinophil viability, density and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-41.
17. Hamid Q, Azzawi M, Ying S, et al. Expression of mRNA for interleukins in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541-6.
18. Mattoli S, Mattoso VL, Soloperto M, Allegra L, Fasoli A. Cellular and biochemical characteristics of bronchoalveolar lavage fluid in symptomatic nonallergic asthma. *J ALLERGY CLIN IMMUNOL* 1991;87:794-802.
19. Walker C, Virchow JC, Bruijnzeel PLB, Blaser K. T-cell subsets and their soluble products regulate eosinophilia in allergic and non allergic asthma. *J Immunol* 1991;146:1829-35.
20. Bjornson BH, Harvey JM, Rose L. Differential effect of hydrocortisone on eosinophil and neutrophil proliferation. *J Clin Invest* 1985;76:924-9.
21. Culpepper JA, Lee F. Regulation of IL-3 expression by glucocorticoids in cloned mucine T-lymphocytes. *J Immunol* 1985;135:3191-7.
22. Friedman B, Schleimer RP. Glucocorticoids inhibit the production of IL-3 and GM-CSF in lectin-stimulated human lymphocytes. *J ALLERGY CLIN IMMUNOL* 1990;85:223.
23. Ryrfeldt A, Andersson P, Edsbacker S, Tonnesson M, Davies D, Pauwels R. Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis* 1982;63(suppl 122):86-95.
24. Toogood JH, Frankish CW, Baskerville JC, Johansson SA, Jennings BH. Mode of action of inhaled budesonide: topical or systemic. In: Hargreave FE, Hogg JC, Malo J-L, Toogood JH, eds. *Glucocorticoids and mechanisms of asthma: clinical and experimental aspects*. Amsterdam: Excerpta Medica Elsevier Science, 1989:105-10.
25. Adelroth E, Rosenhall L, Johansson S-A, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage: the effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990;142:91-9.

26. Frigas E, Gleich GJ. The eosinophil and pathophysiology of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:527-37.
27. Hodges MK, Weller PF, Gerard NP, Ackerman SJ, Drazen JM. Heterogeneity of leukotriene C4 production by eosinophils from asthmatic and from normal subjects. *Am Rev Respir Dis* 1988;138:799-804.
28. Djukanovic R, Wilson JW, Britton KM, et al. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunocytochemistry. *Am Rev Respir Dis* 1990;142:863-71.
29. Bousquet J, Chané P, Lacoste JY, Barneon G, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
30. Frick WE, Sedgwick JB, Busse WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989;139:685-8.

Increased plasma platelet-activating factor in children with acute asthmatic attacks and decreased in vivo and in vitro production of platelet-activating factor after immunotherapy

Kue-Hsiung Hsieh, MD, and Cheng-Koon Ng, BS

Taipei, Taiwan, Republic of China

Background: To explore the possible role of platelet-activating factor (PAF) in the pathogenesis of bronchial asthma, circulating PAF and in vitro production of PAF were studied.

Methods: Radioimmunoassay kits were used in 15 children with acute asthmatic attacks, in 25 newly diagnosed asthmatic children, in 25 good and 18 poor responders to immunotherapy, and in 18 healthy controls.

Results: The results demonstrated the following: (1) PAF was present in the blood of healthy controls. (2) New patients had much higher circulating PAF than did healthy controls ($p < 0.005$), and the circulating PAF decreased after immunotherapy in good ($p < 0.005$) but not in poor responders. (3) The circulating PAF increased up to 20 times that of healthy controls during acute asthmatic attacks. (4) The spontaneous and allergen-stimulated secretion of PAF were markedly increased in new patients and decreased to normal after successful immunotherapy ($p < 0.005$). (5) No increased spontaneous and allergen-stimulated production of PAF was found during acute attacks, but granulocytes from those patients still produced the greatest amount of PAF when stimulated with calcium ionophore A23187. (6) Although a major portion of allergen-induced PAF was secreted, less than 10% of ionophore-induced PAF was secreted.

Conclusion: The findings that the circulating PAF increased markedly during acute asthmatic attacks and the enhanced in vivo and in vitro productions of PAF decreased to normal after successful immunotherapy strongly suggest that PAF may be involved in the pathogenesis of bronchial asthma. (*J ALLERGY CLIN IMMUNOL* 1993;91:650-7.)

Key words: Platelet-activating factor, asthma, immunotherapy, children

From the Division of Allergy, Immunology and Rheumatology, Department of Pediatrics and Graduate Institute of Microbiology and Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.

Supported by a grant from the National Science Council of the Republic of China, grant no. NSC-77-0412-B-002-139.

Received for publication Jan. 14, 1991.

Revised April 2, 1992.

Accepted for publication Oct. 26, 1992.

Reprint requests: Kue-Hsiung Hsieh, MD, Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Rd., Taipei, 10016 Taiwan, Republic of China.

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/43651