

Steroid-resistant asthma: Immunologic and pharmacologic features

Jaime Alvarez, MD, Wendy Surs, BS, Donald Y. M. Leung, MD, PhD, David Iklé, PhD, Erwin W. Gelfand, MD,* and Stanley J. Szeffler, MD
Denver, Colo.

Glucocorticoids play an important role in asthma therapy; however, a subset of patients are poorly responsive. We evaluated immunologic and pharmacologic features of 17 patients with steroid-resistant (SR) asthma (six male and 11 female patients) between the ages of 16 and 69 years (mean age, 29 years). SR asthma was defined as failure to improve morning prebronchodilator FEV₁ >60% predicted after a 2-week course of oral prednisone (mean dose, 45 mg/day). These patients were compared to 24 steroid-sensitive (SS) patients, aged 5 to 70 years (mean age, 17 years; 17 male and seven female patients), and 47 healthy control subjects, aged 20 to 40 years. Mean prednisone dose in SS patients was 25 mg/day. Steroid pharmacokinetics were evaluated in six patients with SR asthma. All studies were within normal limits. Peripheral blood mononuclear cells (PBMCs) from all subjects were stimulated with 10 µg/ml of phytohemagglutinin and incubated for 72 hours with 10⁻⁵ to 10⁻⁹ mol/L of methylprednisolone (Mpn). The Mpn dose-response curve for PBMCs from patients with SR asthma demonstrated a significant ($p < 0.05$) increase in DNA synthesis, that is, more T cell proliferation than PBMCs stimulated with phytohemagglutinin in the presence of Mpn, as compared to SS patients and normal subjects. This augmentation of DNA synthesis was reversible with 10 µg/ml of troleandomycin in vitro. We conclude that PBMCs from patients with SR asthma demonstrate altered response to Mpn in the presence of a T cell mitogen. This abnormality in cellular response may contribute to persistent airway inflammation in patients with SR asthma despite glucocorticoid therapy. Furthermore, this immunologic abnormality appears to be reversible. (J ALLERGY CLIN IMMUNOL 1992;89:714-21.)

Because of their anti-inflammatory properties, GCs have been successfully used in the treatment of asthma since the early 1950s. Despite the usually favorable response, several investigators have described patients with asthma with suboptimal response to GCs.

From the Departments of Pediatrics and Biostatistics, National Jewish Center for Immunology and Respiratory Medicine, The Raymond and Beverly Sackler Foundation, and the Departments of Pediatrics, Pharmacology, and Preventive Medicine and Biometrics, University of Colorado Health Sciences Center, Denver, Colo.

Supported in part by National Heart, Lung, and Blood Institute Grant HL-36577, and by the Food and Drug Administration, Program in Orphan Drugs, Grant FD-R-000278.

Received for publication June 14, 1991.

Revised Oct. 22, 1991.

Accepted for publication Nov. 6, 1991.

Reprint requests: Stanley J. Szeffler, MD, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Rm K926, Denver, CO 80206.

*Dr. Erwin W. Gelfand is a scholar of the Raymond and Beverly Sackler Foundation and was supported in part by National Institutes of Health Grant AI-26490 and AI-29704.

1/1/34893

Abbreviations used

GC:	Glucocorticoid
Mpn:	Methylprednisolone
PBMC:	Peripheral blood mononuclear cell
PHA:	Phytohemagglutinin
SR:	Steroid resistant
SS:	Steroid sensitive
TAO:	Troleandomycin
IL:	Interleukin
k _a :	Elimination rate constant

Schwartz et al.¹ first described a group of six subjects with steroid-dependent asthma who, despite treatment with large doses of oral prednisone, failed to demonstrate improvement of their symptoms and failed to demonstrate Cushingoid features. Carmichael et al.² described a population of patients with chronic asthma who had prebronchodilator FEV₁ of <60% of predicted normal and whose FEV₁ never improved >15% after a 7-day course with 20 mg of oral daily prednisolone. When patients with SR asthma were com-

TABLE I. Patient's characteristics for groups with asthma

Group	Sample size	Age (yr)	Prednisone dose (mg/day)	FEV ₁ (% predicted)
SR	17	29 ± 4.8	44.6 ± 3.8	52.7 ± 1.8
SS	24	17 ± 2.7	24.8 ± 3.0	80.7 ± 2.0

Mean ± SEM.

pared with SS patients (defined as those whose FEV₁ had at least increased by 30% or more at the end of the prednisolone trial), the patients with SR asthma more commonly had a family history of asthma, and a higher percentage had symptoms of asthma for more than 5 years. Patients with SR asthma differed from SS patients in several ways. Their peak expiratory flow rate tended to fall more steeply in the morning, and they were more liable to experience nocturnal or early morning wheeze.

Searching for a cellular mechanism to explain these observations, Poznansky et al.³ found that when PBMCs from patients with SR asthma were stimulated with PHA, colony formation could not be inhibited by 10⁻⁸ mol/L of Mpn as compared to that of SS patients. The investigators attributed this observation to a defect in monocyte response to GCs. Since their initial study, these and other investigators^{4,5} have used PHA-stimulated mononuclear cells incubated with *in vitro* GCs as a method to assess steroid sensitivity.

We conducted studies to determine whether an *in vitro* assay with PHA-stimulated PBMCs could be used to identify cellular abnormalities in GC response in subjects with severe, steroid-requiring asthma. Furthermore, we examined whether the phenomenon of *in vitro* GC resistance, if it were present, was reversible.

MATERIAL AND METHODS

Patient selection

Three patients' groups were selected for evaluation. Patients with SR asthma included 17 (six male and 11 female patients) subjects with severe steroid-dependent asthma between the ages of 16 and 69 years (mean age, 29 years). These patients were labeled clinically SR since their pre-bronchodilator FEV₁ recorded at 6 AM persistently remained <60% of predicted despite oral prednisone doses exceeding 40 mg/day for more than 2 weeks. (Mean prednisone dose was 45 mg/day.) PBMCs from these patients were obtained after the 2-week course of steroids. In six of these patients, a steroid pharmacokinetic evaluation was performed to assess the rate and extent of absorption and metabolism of prednisone and its active metabolite, prednisolone.

SS patients included 24 patients with severe asthma (17 male and seven female patients) with ages ranging from 5 to 70 years (mean age, 17 years). These patients were cat-

egorized as SS based on their response to prednisone. All patients received oral prednisone for at least 7 days (mean prednisone dose, 24.7 mg/day) and had an improvement in their morning prebronchodilator FEV₁ >60% of predicted (Table I).

Concurrent therapy for patients in both groups included inhaled steroids as well as oral and inhaled bronchodilators. The diagnosis of asthma was ascertained by a 20% improvement in FEV₁ after inhaled albuterol on at least one occasion before enrollment in the study.

The control group included 47 healthy adult volunteers, ages ranging between 20 to 40 years, with no history of respiratory disease and not receiving any medication. The study protocol was approved by the National Jewish Center Institutional Review Board. Informed consent was obtained from the patients and normal volunteers. For patients younger than 18 years of age, consent was obtained from the patient and a parent before enrollment in the study. Subjects were excluded if they had any evidence of viral and/or gastrointestinal illness within 3 weeks before blood sampling. Other exclusion criteria included evidence of hepatic or renal disease, smoking, pregnancy, or concomitant administration of drugs known to alter either GC absorption or elimination, such as anticonvulsants or macrolide antibiotics.

Methods

Lymphocyte studies. Peripheral blood (20 ml) was collected in a heparinized syringe (preservative-free heparin, 1000 U/ml) between 6 and 8 AM in all subjects and before administration of GC dose in patients with asthma. Blood samples were obtained at the end of a 1-week treatment period with oral prednisone for SS patients and at the end of 2 weeks for patients with SR asthma. PBMCs were obtained after Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) gradient centrifugation and suspended in RPMI 1640 (Gibco Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum (Hyclone Laboratories, Inc., Logan, Utah), L-glutamine (146 mg/500 ml), penicillin (25000 U/500 ml), streptomycin (25000 µg/500 ml), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (1 mmol/L solution), and sodium pyruvate (100 mmol/L solution). Cells (1 × 10⁵ cells per 200 µl) were plated in 96-well flat-bottom culture plates (Falcon 3072, Beckton Dickinson Labware, Lincoln Park, N.J.). PHA (Sigma Chemical Co., St. Louis, Mo.), 10 µg/ml final concentration, and Mpn (Upjohn Co., Kalamazoo, Mich.) were added to the wells. Mpn was dissolved in sterile water at concentrations ranging from 10⁻⁵

to 10^{-9} mol/L. Cells were incubated for 72 hours at 37°C in water jacketed incubators with 5% CO_2 . Four hours before harvesting, [^3H]thymidine (ICN Biomedicals, Inc., Irvine, Calif.) was added at a final concentration of 1 μCi per well. Cells were then harvested with a PHD cell harvester (Cambridge Technology, Inc., Watertown, Mass.) onto glass-fiber filter disks (Cambridge Technology, Inc.). Disks were transferred to counting vials and dried at room temperature. Isotone solution (Biosafe II solution, Research Products International Corp., Mt. Prospect, Ill.) was then added, and incorporated tritium was counted in a liquid scintillation counter (Beckman LS 5000 TA, Beckman Instruments, Fullerton, Calif.).

After the initial data evaluation, PBMCs from a group of 13 normal volunteers and 14 patients with asthma (five with SR asthma and nine SS) were stimulated with PHA and incubated in vitro with TAO methane sulfonate salt (Pfizer Medical Laboratory, Groton, Conn.) at concentrations ranging from 0.1 to 100 $\mu\text{g}/\text{ml}$ in the presence or absence of Mpn (10^{-7} mol/L) to assess whether in vitro TAO manifests immunosuppressive activity.

GC pharmacokinetics. Patients fasted from midnight and were admitted to the National Jewish Center Pediatric Special Care Unit at 5:30 AM. An indwelling catheter was inserted into a peripheral vein for serial blood withdrawal. At 6 AM, a dose of prednisone (Upjohn Co.), equivalent to 40 $\text{mg}/1.73\text{ m}^2$, was administered orally. One patient received Mpn as maintenance therapy (Upjohn Co.) and was evaluated with this medication. Blood samples (5 ml) were obtained before dose and at 1/2, 1, 1 1/2, 2, 3, 4, 6, 8, 10, and 12 hours after the GC study dose and collected in heparinized tubes. Plasma was separated and stored at -20°C before analysis. Concentrations of prednisolone or Mpn and cortisol were assayed by high-pressure liquid chromatography according to the method of Ebling et al.⁶ The lower limit of detection is 10 ng/ml for these three GCs, and the intraday percent coefficient of variation is $>5\%$ at 50 and 400 ng/ml.

Model independent parameters for prednisolone and Mpn were calculated as follows: The area under the plasma concentration-time curve was determined by LAGRAN⁷ polynomial interpolation and integration with the least-squares terminal slope (k_{el}) to extrapolate to time infinity.

Total body clearance (Cl) was calculated as

$$\text{Cl} = \frac{\text{dose}}{\text{AUC}}$$

The k_{el} was determined from linear regression of the terminal slope of the serum concentration time curve, and half-life was $0.693/k_{el}$. These calculations assume total availability of systemically administered GC, minimal alterations in the degree of reversible metabolism to inactive metabolites, and a linear elimination pattern.

Statistical analysis

In all cases, statistical analyses were performed on responses expressed as the percent of control. Data are summarized as mean $\pm$ standard error, except where it is noted

otherwise. All tests are two-tailed at the 0.05 level of significance.

The experimental design for the examination of the effect of Mpn concentration on PHA-stimulated PBMCs is a parallel-group design with repeated measures on Mpn concentration. Multivariate repeated-measures analysis of variance was used to test for differences between groups, differences between concentrations, and for a group by time interaction with Wilk's lambda criterion. In addition, a priori linear contrasts were used to test for specific differences between groups, and polynomial contrasts were used to examine the relationship between concentration and response. Individual 95% confidence intervals on the mean response to each time were used to determine if specific means were different from 100%.

The experimental design for the analyses of the effect of TAO concentration on lymphocyte stimulation in both the presence and absence of Mpn is a parallel-group design with repeated measures on TAO concentration. Multivariate repeated-measures analysis of variance was used to test for differences between groups, differences between concentrations, and for a group by concentration interaction, separately with and without Mpn.

RESULTS

Effect of Mpn on PBMC stimulation

When PHA-stimulated PBMCs were incubated for 72 hours with increasing concentrations of Mpn (Fig. 1), there was a significant dose-response relationship caused by Mpn concentration ($p \leq 0.0001$) and a significant difference related to study groups ($p = 0.023$), and the group by concentration interaction was not significant ($p = 0.59$). The linear contrast indicated there was not a significant difference between the SS patients and normal subjects ($p = 0.60$), but there was a significant difference between the group with SR asthma and the mean of the other two study groups ($p \leq 0.0001$). Polynomial contrasts indicated these data include components of a linear ($p \leq 0.0001$), quadratic ($p = 0.019$), and cubic ($p = 0.0026$) trend, consistent with a dose-response relationship for the effect of increasing Mpn concentration. It is important to note, however, that one high point in the group with SR asthma, at an Mpn concentration of 10^{-9} mol/L, was a significant outlier. Removal of this subject changed the level of significance for the group effect, making the result not significant at the 0.05 level ($p = 0.095$). The result is significant at the 0.10 level.

At Mpn 10^{-8} mol/L, the degree of inhibition was similar for the SS patients with asthma and normal subjects ($82.6\% \pm 3.5\%$ and $79.6\% \pm 3.3\%$, respectively). Only minimal inhibition was observed in the patients with SR asthma ($97.1\% \pm 7.9\%$). At lower concentrations of Mpn (10^{-9} mol/L), PBMCs from patients with SR asthma demonstrated enhance-

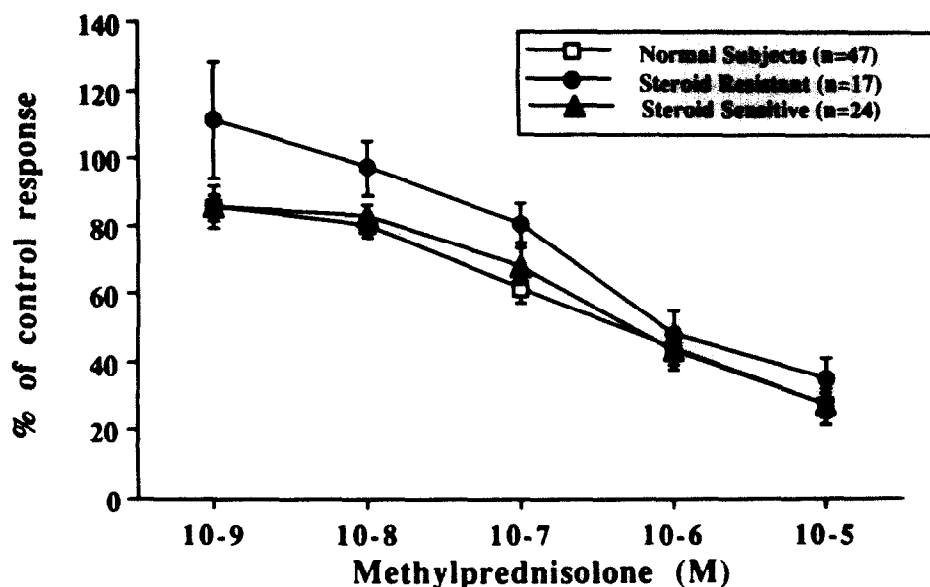


FIG. 1. PHA-stimulated PBMCs from SS patients with asthma demonstrate similar characteristics when they are compared to normal subjects. Significant difference between SR group versus SS and control groups ($p \leq 0.0001$), but no significant difference between SS group and control group ($p = 0.60$). Patients with SR asthma demonstrate increased [^3H]thymidine incorporation when their cells are incubated with low concentrations of Mpn (10^{-9} mol/L) ($p < 0.05$).

ment of DNA synthesis (95% confidence interval, 107.7% to 114.5%). In contrast, cells from SS patients with asthma and normal subjects continued to demonstrate inhibition of [^3H]thymidine uptake at this low Mpn concentration. (Fig. 1).

Effect of TAO on lymphocyte stimulation in vitro

PHA-stimulated PBMCs from 14 patients with asthma (five with SR asthma and nine SS) and 13 normal volunteers were incubated with TAO at concentrations ranging between 1 to 100 $\mu\text{g}/\text{ml}$ in the presence or absence of Mpn (10^{-7} mol/L). Data are summarized in Fig. 2, A, for cells treated with TAO only, whereas data for cells treated with TAO and Mpn are summarized in Fig. 2, B. A significant dose-dependent inhibition of DNA synthesis ($p \leq 0.0001$) was apparent for incubation with TAO alone with no difference in the degree of inhibition noted among the groups ($p = 0.98$) and with no significant interaction between TAO level and group ($p = 0.16$). Similarly, analysis of the TAO and 10^{-7} mol/L of Mpn data demonstrates a significant effect caused by TAO concentration ($p \leq 0.0001$) but no effect caused by group ($p = 0.24$) and no significant interaction between TAO level and group ($p = 0.38$). These results demonstrate that TAO has a significant concentration effect in the absence and presence of Mpn.

The inhibition of DNA incorporation was not due

to cell death because cells whose growth had been inhibited by TAO and Mpn were capable of cellular proliferation once the drugs were removed and cells were restimulated with mitogenic concentrations of PHA. Confirmation of cell viability was also obtained with trypan blue staining.

GC pharmacokinetics in patients with SR asthma

A pharmacokinetic evaluation was performed on six patients with SR asthma, five with oral prednisone and one with Mpn. None demonstrated increased prednisolone or Mpn clearance. Although one patient had a relatively low prednisolone half-life, prednisolone clearance was within the expected range of normal values. Furthermore, the patient receiving Mpn treatment had a decreased clearance rate (Table II).

DISCUSSION

As the inflammatory aspects of asthma become more evident, the use of GC in asthma increases. Despite renewed interest in GC therapy, there are very few ways to assess steroid action. GCs are known to induce neutrophilia by reducing the migration of neutrophils from the vascular compartment.⁸ Serial determinations of peripheral blood cells, including eosinophils, monocytes, and lymphocytes after Mpn administration, have been used as a measure of steroid response.^{1, 9-12} Peripheral lymphocyte counts have

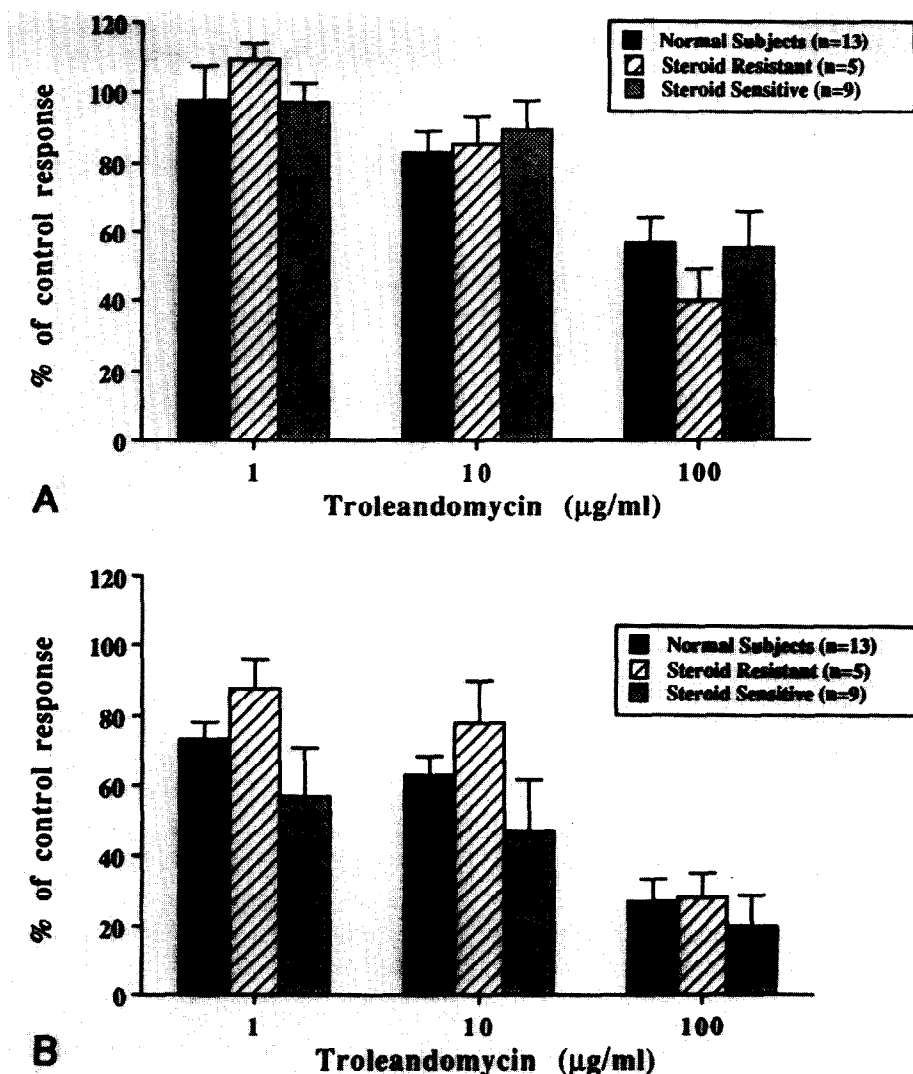


FIG. 2. A, PHA-stimulated PBMCs incubated with varying concentrations of TAO. As TAO concentration is increased, there is a significant decrease in [³H]thymidine incorporation for all three groups tested ($p \leq 0.0001$). B, PHA-stimulated PBMCs incubated with Mpn (10^{-7} mol/L) and varying concentrations of TAO. Addition of Mpn leads to further inhibition in [³H]thymidine incorporation ($p \leq 0.0001$). Results indicate significant TAO concentration effect regardless of whether Mpn is present.

been reported to decrease up to 74% after a single dose of intravenous prednisolone.¹³ Similar findings have been reported in normal volunteers¹⁴ as well as in children with steroid-dependent asthma.¹⁵ Lymphopenia is likely due to redistribution of circulating lymphocytes.⁸ In normal individuals, the administration of GCs causes disappearance of monocytes from the circulation within 6 hours because of sequestration of cells¹⁶ or as a result of decreased output from the bone marrow.¹²

Poznansky et al.³ observed that when PBMCs from SS patients with asthma were placed in soft agar culture plates and stimulated with PHA, colony formation

was not inhibited by Mpn doses as high as 10^{-8} mol/L. This was in contrast to the inhibition demonstrated by Mpn on cells from healthy volunteers. In the absence of Mpn, PBMC colonies from both patients with SR asthma and SS patients with asthma grew in similar numbers. However, growth from the cells of SS patients with asthma was successfully inhibited with concentrations of Mpn as low as 10^{-9} mol/L, whereas PBMCs from patients with SR asthma demonstrated 40% less inhibition when PBMCs were exposed to 10^{-8} mol/L of Mpn. The investigators attributed these observations to a monocyte defect in patients with SR asthma.

TABLE II. Pharmacokinetic data in SR

Patient	Age (yr)	GC	Clearance (ml/min/1.73 m ²)	Half-life (hr)
1	16	Oral prednisone	211	3.1
2	12	Oral prednisone	251	1.9
3	14	Oral prednisone	226	2.6
4	18	Oral prednisone	186	2.8
5	13	Oral prednisone	205	2.6
6	15	Oral Mpn	289	2.9
Kinetic parameters in children with asthma				
Prednisolone (aged 2 to 18 years; N = 16)*			214 ± 58	2.5 ± 0.8
Mpn (aged 9 to 18 years; N = 7)†			381 ± 202	2.34 ± 1.0

Mean ± 2 SD.

*Bartoszek et al. Clin Pharmacol Ther 1987;42:424.

†LaForce et al. J ALLERGY CLIN IMMUNOL 1983;72:34.

Since this study, several investigators^{4,5} have used lymphocyte incorporation of [³H]thymidine as a measure of steroid response. With this approach, our results demonstrate similar findings to previous studies³⁻⁵ in which Mpn concentrations range between 10⁻⁵ to 10⁻⁶ mol/L. At these concentrations, there was no significant difference in the degree of PHA-induced DNA synthesis in PBMCs from patients with SR asthma and SS patients with asthma versus normal subjects, although there was a tendency for less inhibition with PBMCs from patients with SR asthma. When all concentrations were averaged, there was a significant difference between the three study groups, with less inhibitory effects of Mpn on the PBMCs of patients with SR asthma. The differences were particularly apparent at the 10⁻⁹ mol/L of Mpn concentration. In fact, at this concentration of Mpn, there was apparent enhancement of DNA synthesis in patients with SR asthma not observed in the other groups studied.

Mechanisms of GC inhibition of lymphocyte proliferation are poorly understood but are possibly related, in part, to their ability to decrease IL-1 and IL-2 production¹⁷⁻²¹ and to decrease IL-2 receptor expression.²² Activated cells have also been reported to be somewhat refractory to the inhibition of interleukin production induced by GCs.²³ This latter observation may explain the relative lack of inhibition by Mpn on PBMCs derived from patients with SR asthma. An alternative explanation is that continued use of high-dose GCs in patients with SR asthma may lead to steroid-receptor down regulation. This finding has been described in the mouse²⁴ and in human lymphocytes.²⁵ Receptor down regulation could lead to an increase in steroid-dose requirement by making the

cells less sensitive to steroids. Another possible mechanism for decreased steroid responsiveness could be due to alteration of the hypothalamic-pituitary-adrenal axis. Iatrogenic GC-receptor resistance has been described after the use of RU 38486, a potent GC-receptor blocker. Long-term administration of this compound has led normal individuals to activate the hypothalamic-pituitary-adrenal axis, resulting in a resetting of this system at a higher threshold with decreased sensitivity to dexamethasone.²⁶

In our system, the stimulatory response of PBMCs from patients with SR asthma to PHA in the presence of Mpn (10⁻⁹ mol/L) was modified by the addition of TAO in vitro. Incubation with Mpn and TAO in vitro produced a shift in the response to low Mpn concentrations, such that there was no difference between the response in patients with SR asthma and SS patients with asthma. In an effort to determine the effects of orally administered TAO on mitogen response, blood samples were collected from five steroid-dependent patients with asthma (mean age, 14 years; mean prednisone dose, 27 mg/day; mean FEV₁, 77%) participating in a 3-month, double-blinded, randomized trial with oral TAO plus an oral GC (either prednisone or Mpn) compared to Mpn alone. These patients were selected because their asthma symptoms could not be controlled with conventional therapy. PBMCs from these patients were obtained before initiating the trial, at 2 weeks, and at 3 months. Cells were stimulated with PHA and incubated in the absence and presence of Mpn (10⁻¹⁰ mol/L). In five patients who received TAO therapy, there was enhancement of DNA incorporation (109.6% ± 27.6%) before TAO therapy, but when PBMCs were examined at 2 (72.8% ± 14.6%) and 12 weeks

(73.6% $\pm$ 13.3%) after TAO therapy, there was a trend toward decreased DNA incorporation compared to pretreatment measures (unpublished observations). Four of the five patients demonstrated clinical improvement while they were receiving TAO therapy, and three patients tolerated a significant reduction in steroid dose.

These findings suggest that TAO, a macrolide antibiotic, has potential immunomodulatory properties. Perhaps the beneficial clinical effects observed with combined Mpn and TAO therapy^{27, 28} may not only be related to inhibition of Mpn metabolism,^{29, 30} but possibly to independent immunomodulatory properties of TAO. The use of TAO and erythromycin has been associated with decreased airway hyperresponsiveness.^{28, 31} Other structurally related macrolide antibiotics, FF 506 and rapamycin, have profound immunomodulatory effects on murine T cells. FK 506 alters calcium-associated events of T cell activation and blocks T cell proliferation by suppressing lymphokine production. Rapamycin inhibits T cells by impairing their response to growth-promoting lymphokines.³²

It is unlikely that short GC courses significantly alter PBMC response to Mpn in vitro, even at low concentrations. The group of 24 SS patients with asthma who received oral prednisone in excess of 25 mg/day for 2 weeks did not manifest enhancement of proliferation when their cells were exposed to Mpn concentrations of 10^{-9} mol/L. At that concentration, cells from these patients continued to demonstrate inhibition similar to normal volunteers.

Multiple mechanisms likely explain the poor response to GC therapy in patients with SR asthma. Although steroid resistance in certain patients with asthma may be attributed to impaired GC absorption or rapid elimination, this was not observed in the six patients with SR asthma in whom a pharmacokinetic evaluation was performed. This finding confirms previous observations in a similar group of patients with SR asthma⁵ and indicates that other mechanisms must be sought to explain poor clinical response in these patients. Nevertheless, an evaluation of GC pharmacokinetics in these patients is useful since certain individuals may have reduced absorption or rapid elimination.³³ An adjustment in dose, substitution of an alternative GC, or stopping a medication responsible for increasing GC metabolism, for example certain anticonvulsants, could result in improved clinical response.

It is intriguing to pursue the possibility of an abnormal cellular response to GCs as a potential mechanism for ongoing inflammation. Our observations support the hypothesis that a PHA-stimulation test in

PBMCs may be helpful in discerning between patients with SR asthma and SS patients. Perhaps other markers of T cell activation will provide greater sensitivity. Additional studies are also needed to verify the presence of persistent inflammation in the airways of patients with SR asthma. A readily available marker of inflammation would be extremely useful in identifying patients who are poorly responsive to GC therapy and assist in the evaluation of alternative treatments.

We thank William F. Kastner for performing the statistical analysis.

REFERENCES

- Schwartz HJ, Lowell FC, Melby JC. Steroid resistance in bronchial asthma. *Ann Intern Med* 1968;69(3):493-9.
- Carmichael J, Paterson IC, Diaz P, Crompton GK, Kay AB, Grant IWB. Corticosteroid resistance in chronic asthma. *Br Med J* 1981;282:1419-22.
- Poznansky MC, Gordon ACH, Grant IWB, Wyllie AH. A cellular abnormality in glucocorticoid-resistant asthma. *Clin Exp Immunol* 1985;61:135-42.
- Wyllie AH, Poznansky MC, Gordon ACH. Glucocorticoid-resistant asthma: evidence for a defect in mononuclear cells. In: Kay AB, ed. *Asthma clinical pharmacology and therapeutic progress*. Oxford: Blackwell Scientific, 1986:306-14.
- Corrigan CJ, Brown PH, Barnes NC, et al. Glucocorticoid resistance in chronic asthma. Glucocorticoid pharmacokinetics, glucocorticoid receptor characteristics, and inhibition of peripheral blood T cell proliferation by glucocorticoid in vitro. *Am Rev Respir Dis* 1991;144:1016-25.
- Ebling WF, Szefer SJ, Jusko WJ. Analysis of cortisol, methylprednisolone, and methylprednisolone hemisuccinate: absence of effects of troleandomycin on ester hydrolysis. *J Chromatogr* 1984;305:271-80.
- Rocci ML, Jusko WJ. LAGRAN program for area and moments in pharmacokinetic analysis. *Comput Programs Biomed* 1983;3:203-16.
- Haynes BF, Fauci AS. The differential effect of in vivo hydrocortisone on the kinetics of subpopulations of human peripheral blood thymus-derived lymphocytes. *J Clin Invest* 1978;61:703-7.
- Rinehart JJ, Balcerzak SP, Sagone AL, LoBuglio AF. Effects of corticosteroids on human monocyte function. *J Clin Invest* 1974;54:1337-43.
- Rinehart JJ, Sagone AL, Balcerzak SP, Ackerman GA, LoBuglio AF. Effects of corticosteroid therapy on human monocyte function. *N Engl J Med* 1975;292:236-41.
- Dale DC, Fauci AS, Guerry D, Wolff SM. Comparison of agents producing a neutrophilic leukocytosis in man: hydrocortisone, prednisone, endotoxin, and ethiocholanone. *J Clin Invest* 1975;56:808-13.
- Thompson J, Van Furth R. The effect of glucocorticosteroids on the proliferation and kinetics of promonocytes and monocytes of the bone marrow. *J Exp Med* 1973;137:10-21.
- Coburg AJ, Gray SH, Katz FH. Disappearance rates and immunosuppression of intermittent intravenously administered prednisolone in rabbits and human beings. *Surg Gynecol Obstet* 1970;131:933-42.
- Yu DTY, Ramer SJ, Clements PJ. Effect of methylprednisolone on autologous mixed lymphocyte cultures. *Transplantation* 1978;25:163-5.
- Chai H, Gilbert MT. The effect of alternate-day prednisone on

- the white blood count in children with chronic asthma. *J ALLERGY CLIN IMMUNOL* 1973;51:65-70.
16. Thompson J, Van Furth R. The effect of glucocorticoids on the kinetics of mononuclear phagocytes. *J Exp Med* 1970;131:429-42.
 17. Gillis S, Crabtree GR, Smith KA. Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. *J Immunol* 1979;123:1624-31.
 18. Snyder DS, Unanue ER. Corticosteroids inhibit murine macrophage Ia expression and interleukin-1 production. *J Immunol* 1982;129:1803-5.
 19. Kern JA, Lamb RJ, Reed JC, Daniele RP, Nowell PC. Dexamethasone inhibition of interleukin-1 beta production by human monocytes. *J Clin Invest* 1988;81:237-44.
 20. Crabtree GR, Munk A, Smith KA. Glucocorticoids inhibit expression of Fc receptors on the human granulocytic cell line HL-60. *Nature* 1979;279:338-9.
 21. Kaplan MP, Lysz K, Rosenberg SA, Rosenberg JC. Suppression of interleukin-2 production by methylprednisolone. *Transplant Proc* 1983;15:407-10.
 22. Horst HJ, Flad HD. Corticosteroid-interleukin-2 interactions: inhibition of binding of interleukin-2 to interleukin-2 receptors. *Clin Exp Immunol* 1987;68:156-61.
 23. Piccolella E, Vismara D, Lombardi G, Guerritore D, Piantelli M, Ranelletti FO. Effect of glucocorticoids on the development of suppressive activity in human lymphocyte response to a polysaccharide purified from *Candida Albicans*. *J Immunol* 1985;134(2):1166-71.
 24. Vedeckis WV, Ali M, Allen HR. Regulation of glucocorticoid receptor protein and mRNA levels. *Cancer Res* 1989;49:2295s-2302s.
 25. Rosewicz S, McDonald AR, Maddux BA, Goldfine ID, Miesfeld RL, Logsdon CD. Mechanism of glucocorticoid receptor down-regulation by glucocorticoids. *J Biol Chem* 1988;263:2581-4.
 26. Lamberts SWJ, Koper JW, den Holder FH, de Jong FH. Familial and iatrogenic receptor resistance. *Cancer Res* 1989;49:2217s-9s.
 27. Itkin IH, Menzel ML. The use of macrolide antibiotic substances in the treatment of asthma. *J ALLERGY* 1970;45(3):146-62.
 28. Spector SL, Katz FH, Farr RS. Troleandomycin: effectiveness in steroid-dependent asthma and bronchitis. *J ALLERGY CLIN IMMUNOL* 1974;54(6):367-79.
 29. Szeffler SJ, Rose JQ, Ellis EF, Spector SL, Green AW, Jusko WJ. The effect of troleandomycin on methylprednisolone elimination. *J ALLERGY CLIN IMMUNOL* 1980;66(6):447-51.
 30. Szeffler SJ, Brenner M, Jusko WJ, Spector SL, Flesher KA, Ellis EF. Dose- and time-related effect of troleandomycin on methylprednisolone elimination. *Clin Pharmacol Ther* 1982;32:166-71.
 31. Miyatake H, Taki F, Taniguchi H, Suzuki R, Takagi K, Satake T. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 1991;99:670-3.
 32. Dumont JF, Staruch MJ, Koprak SL, Melino MR, Sigal NH. Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. *J Immunol* 1990;144:251-8.
 33. Hill MR, Szeffler SJ, Ball BD, Bartoszek M, Brenner AM. Monitoring glucocorticoid therapy: a pharmacokinetic approach. *Clin Pharmacol Ther* 1990;48:390-8.

AVAILABLE NOW! The FIVE-YEAR (1981-1985) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$42.00. This comprehensive, 156-page reference guide is a current presentation of all topics included in the *JOURNAL* from January 1981 to December 1985 (volumes 67-76)—the past 10 volumes. It incorporates complete references to over 672 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1,525 Subject Headings, under which there are 5,316 references. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 4,780 Author Entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: Mosby-Year Book, Inc., 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE 1-800-325-4177, Subscription Services, ext. 4351, or 314-453-4351. Please reference book code number 2605-6 when placing your order. **PREPAYMENT REQUIRED.** Make checks payable to Mosby-Year Book, Inc. (All payments must be in US funds drawn on a US bank.) Price: \$42.00 in the US, \$44.75 international (price includes mailing charges).