

The effects of upper respiratory infection on T-cell proliferation and steroid sensitivity of asthmatics

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Background: The common cold is a potent trigger for asthma symptoms. Although the mechanisms are not completely understood, inflammatory events may play an important role.

Objective: We tested the hypothesis that changes in steroid responsiveness are implicated in the relationship between asthma and the common cold.

Methods: We investigated the steroid sensitivity of T cells from 10 patients with asthma (not taking steroids) and 10 control subjects, during upper respiratory infection and at recovery (6 weeks later). We stimulated peripheral blood mononuclear cells with phytohemagglutinin to measure T-cell proliferation, sensitivity to the inhibitory effects of steroids, and cytokine production.

Results: During infection compared with recovery, the asthma group exhibited lower responsiveness to dexamethasone at 10^{-10} and 10^{-9} mol/L and to 10^{-10} mol/L hydrocortisone ($P < .05$) and both groups had decreased T-cell proliferation ($P < .05$). During recovery, patients with asthma had greater T-cell proliferation than control subjects ($P < .02$); the values expressed as uptake of [^{3} H]-thymidine were 60119 ± 3944 cpm (asthma) and 39078 ± 6459 cpm (control subjects). The asthma group had higher TNF- α and IFN- γ production during infection ($P < .05$); changes in IL-8 and IL-10 levels were not significant, and IL-4 was not detected.

Conclusion: During infection, asthmatic subjects exhibit decreased T-cell responsiveness to steroids. The T-cell proliferative response to phytohemagglutinin, higher in asthma, is decreased with a common cold. These findings are associated with changes in the cytokine profile. Thus viral-induced upper respiratory infections can produce an inflammatory milieu with altered steroid function and selective increases in cytokines. (*J Allergy Clin Immunol* 1998;102:592-7.)

Key words: Asthma, glucocorticoid, T lymphocytes, common cold, phytohemagglutinin

Upper respiratory infections that commonly cause no major problems in the normal host may have profound effects on the lower airway in certain individuals. Of considerable interest is the effect of respiratory infections on people with existing asthma.¹ Nicholson et al,² studying

Abbreviations used

FEF₂₅₋₇₅: Forced expiratory flow between 25% to 75% of the vital capacity

GR: Glucocorticoid receptor

PEFR: Peak expiratory flow rate

URI: Upper respiratory infection

adults with asthma, found that 80% of subjective asthma exacerbations occurred with symptomatic colds and that 89% of symptomatic colds were associated with symptoms of asthma. Although recognized that the common cold is a potent trigger for symptoms of asthma, the mechanisms underlying the association between upper respiratory infection (URI) and increased lower airway obstruction remain obscure. Experimental infection with rhinovirus causes a prolonged increase in the maximal degree of airway narrowing in response to bronchoconstrictor stimuli in asthma.³ These findings are in agreement with results that show an increase in sensitivity to inhaled histamine after inoculation with rhinovirus in subjects with allergic rhinitis.⁴ Busse⁵ and Calhoun et al⁷ showed that viral infection not only increases airway responsiveness but also changes the pattern of allergic airway response. During rhinovirus infection, patients exhibit an increase in airway reactivity and have a greater likelihood of the development of a late asthmatic reaction after exposure to allergen. The available data suggest that URIs alter the intensity of factors that determine and regulate the inflammatory response.

Decreased response to glucocorticoid therapy is an important issue in the treatment of some patients with asthma (ie, steroid-resistant asthma). Asthma appears to be associated with a spectrum of steroid responsiveness in which steroid-resistant asthma is one extreme.⁸ This, in fact, can be seen on a circadian basis in nocturnal asthma patients where decreased steroid responsiveness occurs at night.⁹ Thus we hypothesized that changes in steroid responsiveness (ie, a temporary steroid refractoriness) could be part of the pathophysiologic features of the virus-induced asthma. To assess the steroid sensitivity of patients with asthma during infection, we measured the effects of hydrocortisone and dexamethasone on the T-cell proliferative response to PHA. Also, T-cell proliferation, itself a marker of T-cell activation, and cytokine concentrations were analyzed to improve our understanding of the relationship among asthma, URI, and steroid sensitivity.

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TABLE I. Subject characteristics

	Asthma group	Control group
No of subjects	10	10
Gender (M/F)	4/6	3/7
Age (yrs)	33 ± 1.8	36 ± 2.3
FEV ₁ during infection (%)	88.0 ± 2.9	98.8 ± 3.2
FEV ₁ 6 weeks after infection (%)	91.5 ± 3.7	99.2 ± 3.3
PEFR during infection (L/s)	9.73 ± 1.37	10.07 ± 1.88
PEFR 6 weeks after infection (L/s)	10.81 ± 1.58*	10.33 ± 1.24
FEF ₂₅₋₇₅ during infection (%)	62.1 ± 10	90.0 ± 7.5
FEF ₂₅₋₇₅ 6 weeks after infection (%)	72.5 ± 10*	90.5 ± 5.3
Cold manifestations		
Cough	7/10	5/10
Nasal symptoms†	10/10	10/10
Throat symptoms‡	4/10	4/10
Systemic symptoms§	6/10	5/10

Data are expressed as mean ± SEM.

**P* < .03 compared with infection.

†Nasal drainage and sneezing.

‡Hoarseness and scratchy throat.

§Fever, muscle ache, and headache.

METHODS

Ten patients with asthma and 10 normal volunteers with a URI diagnosed by medical history and physical examination were recruited from the general Denver community. The presence of at least 2 of the following symptoms was necessary for inclusion: cough, headache, hoarseness, scratchy throat, muscle ache, nasal drainage, sneezing, and oral temperature greater than 37.7°C (Table I). Additionally, all subjects had at least 1 of the nasal symptoms (drainage or sneezing). To avoid the autumn and spring allergy seasons, subjects were enrolled between December 1, 1996, and March 14, 1997. Subjects who met diagnostic criteria for asthma¹⁰ were maintained on β₂ agonist and had exhibited a methacholine PC₂₀ of less than 8 mg/mL and/or FEV₁ improvement by inhaled β₂ agonist (0.18 mg albuterol) greater than 15% in the previous 6 months. Both atopic and nonatopic patients with asthma were enrolled, and normal subjects were nonatopic with FEV₁ values of at least 90% predicted. Exclusion criteria included a history of cigarette smoking, use of cromolyn sodium, use of inhaled or oral corticosteroid, use of nonsteroidal antiinflammatory drug within the previous 2 days, immunotherapy within the previous 3 months, and significant nonasthma pulmonary disease or other medical illnesses. Informed consent was obtained from all subjects for this institutional review board-approved protocol.

Subjects came to our laboratory in the morning, within 1 week after the cold symptoms had started and 6 weeks later. At the time of the first visit, the duration of symptoms was 3.6 ± 0.7 days (mean ± SEM) in the asthma group and 3.3 ± 0.6 days in the control group. On both visits, a history review, physical examination, lung function test, and blood-drawing were performed.

Cell culture

Twenty milliliters of heparinized blood was taken from each individual, and PBMCs were isolated by a standard technique of density gradient centrifugation with the use of Ficoll-Paque (Pharmacia, Inc, Piscataway, NJ). The cells were washed 3 times in phosphate-buffered saline and resuspended at a concentration of 1 × 10⁶ cells/mL in RPMI 1640 (GIBCO Labs, Grand Island, NY) supplemented with 10% fetal bovine serum, L-glutamine (4 mmol), peni-

cillin (100 U/mL), and streptomycin (100 μg/mL). The cells were cultured in triplicates with or without added PHA (5 μg/mL) in the presence or absence of increasing concentrations (10⁻¹⁰ to 10⁻⁵ mol/L) of hydrocortisone or dexamethasone (Sigma Chemical Co, St Louis, Mo). The cell cultures were incubated for 72 hours in a humidified atmosphere with 5% carbon dioxide at 37°C.

Lymphocyte proliferation assay

Cell proliferation was measured by uptake of tritiated thymidine. Sterile [³H]thymidine (ICN, Irvine, Calif) was added at a final concentration of 5 μCi/mL. After an additional 6-hour incubation, cells were harvested onto glass-fiber filter paper with a cell-harvester apparatus, and the incorporated radiolabel was counted with a β-spectrometer. The mean of the triplicate samples was expressed as a percentage of the positive control (ie, cells stimulated with PHA in the absence of steroids). The value of background proliferation, that is the uptake by nonstimulated cells (negative control), was subtracted from the measurements.

Cytokine assays

Supernatants from PBMC cultures, stimulated and nonstimulated, were collected and stored at -70°C for cytokine measurements. The concentrations of TNF-α, IFN-γ, IL-4, IL-8, and IL-10 were determined in these supernatants by a quantitative sandwich enzyme immunoassay according to the manufacturer's instruction (R&D Systems Inc, Minneapolis, Minn). The limit of detectability of these assays was 2 pg/mL for TNF-α, IL-8, IL-4, and IFN-γ and 100 pg/mL for IL-10. Samples that registered above the standard curve were diluted and reanalyzed. Levels of cytokines were expressed as picograms per milliliter of supernatant.

Statistical analysis

Proportion of male and female patients and clinical manifestations in the 2 groups were compared with a Chi-squared test. Data were analyzed by Student's unpaired *t* test for comparisons between groups and 2-tailed Student's paired *t* test for intragroup differences (infection to recovery). Cytokine concentrations were also analyzed

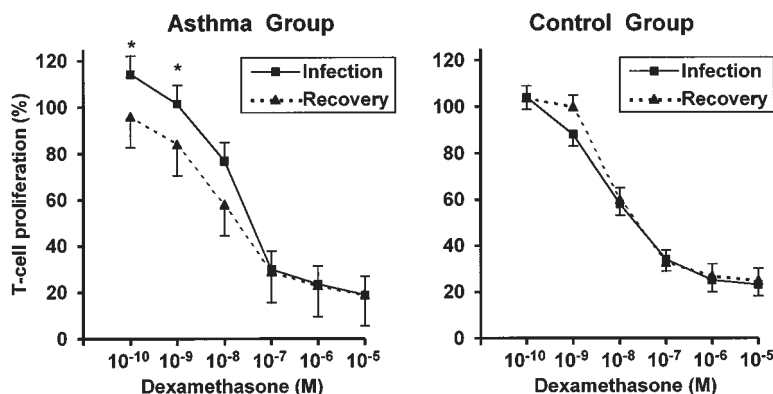


FIG 1. Effects of dexamethasone on PHA-induced proliferation of peripheral blood T cells from subjects with asthma (*asthma group*) and normal subjects (*control group*) during URI (*solid line*) and recovery (*dotted line*). Proliferation (mean $\pm$ SEM) is expressed as a percentage of the proliferation obtained in the absence of any steroid (positive control). * $P < .05$ compared with the recovery phase.

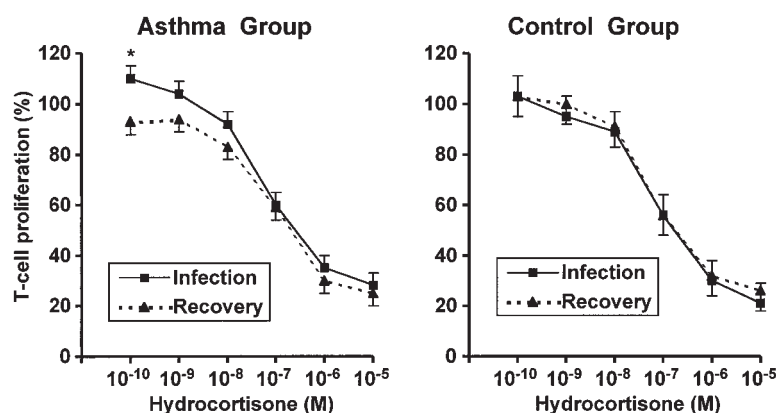


FIG 2. Effects of hydrocortisone on PHA-induced proliferation of peripheral blood T cells from subjects with asthma (*asthma group*) and normal subjects (*control group*) during URI (*solid line*) and recovery (*dotted line*). Proliferation (mean $\pm$ SEM) is expressed as a percentage of the proliferation obtained in the absence of any steroid (positive control). * $P < .05$ compared with the recovery phase.

by ANOVA.¹¹ Data were expressed as mean $\pm$ SEM. A P value $< .05$ was considered significant.

RESULTS

Subject characteristics and lung function values are shown in Table I. There were no differences in demographic parameters between asthma and control groups. Also, the clinical manifestations of URI were similar, and all subjects had nasal drainage and/or sneezing (Table I). Seven patients in the asthma group reported asthma worsening with increased need for the bronchodilator linked to the infection. In addition, FEF₂₅₋₇₅ and PEFR were decreased significantly by the infection (Table I).

As shown in Figs 1 and 2, during infection, T cells from the asthma group were less responsive to the inhibitory effects of dexamethasone at the concentrations of 10^{-10} to 10^{-9} mol/L and hydrocortisone at 10^{-10} mol/L. For the control group, the infection phase was not associated with significant changes in responsiveness to dexamethasone or hydrocortisone (Figs 1 and 2). No dif-

ferences were found between asthma and control groups.

The analysis of T-cell proliferation in PBMC cultures that received no steroid revealed significant differences in both groups from infection to recovery (Fig 3). The mean $\pm$ SEM of the asthma group T-cell proliferation expressed as uptake of [³H]-thymidine (counts per minute) was 38347 ± 7120 cpm in the infection phase and 60119 ± 3944 cpm in the recovery ($P < .05$). In the control group, the same parameter increased from 28507 ± 6250 cpm (infection) to 39078 ± 6459 cpm (recovery; $P < .03$). In addition, the comparison between asthma group and control group T-cell proliferation unveiled a significant difference in the recovery timepoint ($P < .02$).

TNF- α , IFN- γ , and IL-10 levels are shown in Fig 4. Levels of TNF- α decreased from 1522 ± 230 pg/mL (infection) to 1191 ± 264 pg/mL (recovery) in the asthma group ($P < .03$) and varied from 680 ± 104 pg/mL to 865 ± 180 pg/mL in the control group from infection to recovery. The difference between asthma and control TNF- α levels was significant only in the infection phase

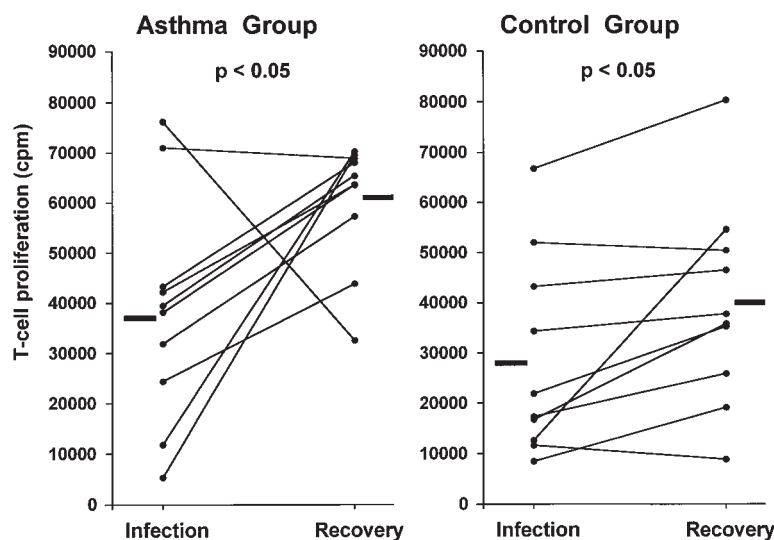


FIG 3. Proliferation of peripheral blood T cells from subjects with asthma (*asthma group*) and normal subjects (*control group*) during URI and recovery. Proliferation is expressed as incorporation of [^{3} H]-thymidine (cpm); $P < .05$ refers to the comparison between infection and recovery within group.

($P < .04$). An analogous behavior was detected for IFN- γ variations. Higher IFN- γ levels in infection (125 ± 47 pg/mL) than in convalescence (40 ± 21 pg/mL) were seen for the asthma group ($P < .05$), and comparable levels were found in both conditions for the control group (31 ± 31 pg/mL and 18.5 ± 18 pg/mL). IL-10 levels were not significantly different between groups or within groups, although the control group infection-related IL-10 concentration was smaller, 1600 ± 1000 pg/mL, compared with 4500 ± 2500 pg/mL during recovery. The asthma group had 5728 ± 1200 pg/mL and 5312 ± 700 pg/mL IL-10 in infection and recovery, respectively. Concentrations of IL-8 in the PBMC cultures of patients with asthma and control subjects during infection and recovery were similar, 172 ± 31 ng/mL and 161 ± 18 ng/mL in the asthma group and 100 ± 24 ng/mL and 164 ± 26 ng/mL in the control group, infection and recovery, respectively. The method was not able to detect IL-4 in any cell culture supernatant. The lower limit of detection was 2 pg/mL. To confirm the ability of the method to measure IL-4 in these supernatants, IL-4 was added to some cell cultures. This added IL-4 was detected by ELISA.

DISCUSSION

The results of this study suggest that, during URI, subjects with asthma (not taking steroids) have decreased T-cell responsiveness to corticosteroids. The effect of steroids on PHA-induced T-cell proliferation was diminished in patients with asthma during infection compared with recovery. This difference reached statistical significance for both steroids tested, hydrocortisone and dexamethasone, at low concentrations. The steroid responsiveness in the control group did not change. In addition

to the altered steroid responsiveness, this study demonstrated decreased T-cell proliferative response to PHA during the URI phase. This decrease was significant for both the asthma and control groups.

At recovery, the analysis revealed that patients with asthma have heightened T-cell proliferation compared with control subjects (60119 cpm versus 39078 cpm, respectively), suggesting a chronic situation with more T-cell activation. Increased T-cell activation in the peripheral blood of patients with asthma had been previously reported and shown to be correlated with asthma severity.^{12,13} Possible explanations for our finding are a "spillover" of T cells activated from the bronchial mucosa and/or an influence of the asthmatic bronchial microenvironment on circulating T lymphocytes.¹⁴

The cytokine profile demonstrated higher levels of TNF- α production by cells from asthmatic subjects compared with cells from control subjects at both timepoints. The comparison between infection and recovery showed greater TNF- α levels during infection only in the asthma group. In a similar fashion, the production of IFN- γ had a 3-fold augmentation related to infection in the asthma group with no significant change for the control group. The variation in IL-10 measurements, lower in control subjects during infection, did not reach statistical significance. The production of IL-8 was comparable during infection and recovery in both groups. IL-4 was not present in detectable amounts for any of the samples examined.

The relevance of suppressing T-cell proliferation in vitro as a measure of clinical steroid sensitivity has been addressed by several authors who perform asthma research. PBMCs from patients with asthma that is clinically resistant to oral glucocorticoid therapy are rela-

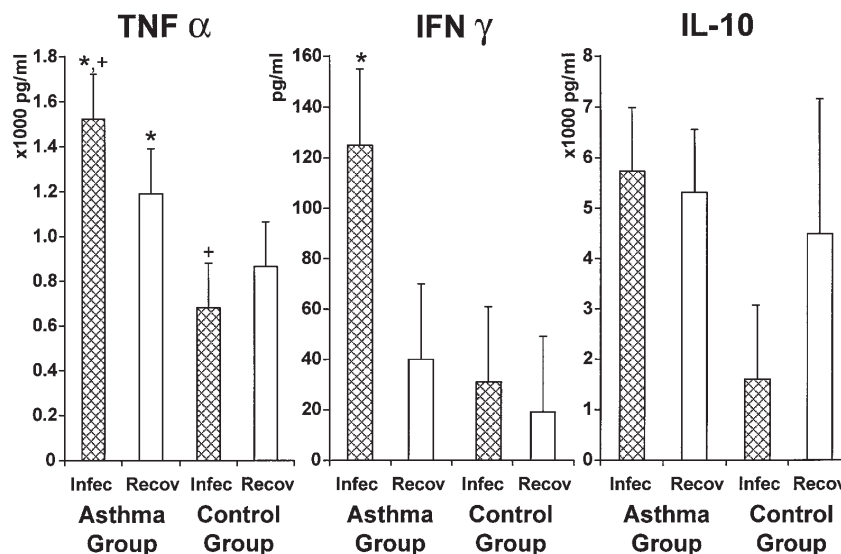


FIG 4. Concentrations of TNF- α , IFN γ , and IL-10 in the culture of PHA-stimulated PBMC from subjects with asthma (*asthma group*) and normal subjects (*control group*) during URI (*cross-hatched bars*) and recovery (*open bars*). Data are expressed as mean $\pm$ SEM. *Significant difference ($P < .05$) between infection and recovery (within group). +Significant difference ($P < .05$) between asthma group and control group.

tively insensitive to the antiproliferative effects of glucocorticoids *in vitro*.^{15,16} Furthermore, published data indicate that the method used in this study is highly reproducible in terms of coefficient of variation of repeated analysis on the same subject and in the long term if patients are retested over a period of time.¹⁷ A significant correlation between blood cells and lung cells alterations in steroid responsiveness has been demonstrated in our laboratory by studying nocturnal asthma.⁹

The mechanisms that control sensitivity to steroids are not fully understood. The studies on steroid-resistant asthma provide some information on the variables that regulate responsiveness to corticosteroids. Peripheral blood lymphocytes isolated from glucocorticoid-resistant asthma and activated with PHA are more refractory to a variety of glucocorticoid compounds compared with those from sensitive patients.¹⁸ This suggests that glucocorticoid insensitivity does not depend on the precise structure of the molecule itself. The similarity of results obtained with hydrocortisone and dexamethasone in this study fits into this theory. Reduction in glucocorticoid receptor (GR)-binding affinity has been reported in steroid-resistant asthma. This abnormality may be secondary to cytokine exposure, because the normalization of GR-affinity *in vitro* is prevented by a combination of IL-2 and IL-4, and this combination reduces the GR-affinity in T lymphocytes from normal donors,^{19,20} which suggests that steroid resistance may occur in the airways of patients with asthma as a secondary phenomenon caused by the local production of cytokines. Recently, allergen exposure was shown to reduce GR-affinity in patients with atopic asthma. The mechanism for this alteration also appears to be due to the production of IL-2 and IL-4 after allergen stimulation.²¹ The cytokine

measurements support the theory of increased inflammation during infection in our patients with asthma. However, IL-4 concentrations were not detectable in our study, which may indicate that a different cytokine profile is associated with steroid insensitivity in viral infections. It is also possible that steroid insensitivity may evolve as a result of transcription factor activation by TNF- α ^{22,23} or a direct consequence of viral infection.²⁴

The cytokine results indicate a T_{H1}-like response, which has been described to be frequently associated with viral infections. T_{H1} cells are characterized by secretion of IFN- γ and IL-2; IFN- γ has direct and indirect antiviral activity. T_{H2} cells selectively produce IL-4 and IL-5²⁵; and certain pathogens, such as respiratory syncytial virus infection in infants, leads to this predominant T_{H2}-like cytokine response.²⁶

Clinical relevance of the reported difference in sensitivity may be limited by the fact that this alteration was not found at high steroid concentrations commonly achieved in serum by administering therapeutic dosages, although more severe asthma or infection may induce the biggest shift in the dose response curve toward steroid refractoriness. A particular infection may cause greater or lesser variations in this issue. Considering that rates of virus definition during a URI are relatively low, especially in adults, in this initial study we did not perform pathogen identification.² Further studies should include more severe asthma, monitoring of small-airway function, experimental infection, analysis of lung cells, and measurement of cytokines in bronchoalveolar lavage or tissue to definitely incorporate the recorded phenomenon to the mechanisms of virus-induced asthma.

In conclusion, patients with asthma, who have a URI, exhibit decreased T-cell responsiveness to steroids. The

T-cell proliferative response to PHA, higher in asthma, is decreased by the common cold. These findings are associated with changes in the cytokine profile. Thus viral-induced URIs can produce an inflammatory milieu with altered steroid function and selective increases in cytokines.

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