T Cell Profile After Systemic Steroid Burst in Inner-City Asthmatic Children with Recurrent Infections

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Background: Systemic corticosteroids are the standard of care for acute asthma exacerbation. Respiratory infections are known as common triggers of asthma exacerbation, but the risk of immune suppression from frequent periodic use of systemic steroids in poorly controlled asthmatic children is not well studied.

Materials and Methods: We conducted a retrospective chart review of 26 children, 3–15 years old with poorly controlled, moderate-to-severe persistent asthma who received ≥ 2 systemic corticosteroid/year. The data collected include absolute T cell, B cell, and natural killer (NK) cell counts; lymphocyte proliferation studies to phytohemagglutinin (PHA), concanavalin A (CON A), and pokeweed mitogen; immunoglobulin G and M; and antibody titers to tetanus, diphtheria, and pneumococcus. Frequency tables and crosstabs were used to analyze the data.

Results: Low CD4⁺ T cell counts were found in 47.8% of the patients, and 45.8% had low CD3⁺ T cell counts. The lymphocyte proliferation studies data exhibited variability, but 21.4%–75% of the subjects who demonstrated normal T cell counts had decreased lymphocyte proliferation studies to PHA and CON A. All the patients had normal immunoglobulins, B cell, and NK cell counts. All but 1 patient had adequate antibody responses to *Streptococcus pneumoniae*.

Conclusions: Frequent systemic corticosteroid use may suppress T cell number and function in asthmatic children. This can potentially lead to increase susceptibility for future infections and asthma exacerbations. Depressed lymphocyte proliferations are observed even in patients who demonstrated normal T cell counts. This emphasizes the importance of adherence to asthma controller medications, and control of asthma triggers, to limit the frequency of steroid use.

Keywords: corticosteroids, acquired immunodeficiency, asthma exacerbation, asthma in children, inner-city asthma

Introduction

APPROXIMATELY 6.2 MILLION CHILDREN in the United States have asthma. Asthma is the leading cause of missed school days¹ and a significant cause of mortality in children. In 2015, 219 children younger than 18 years of age died from asthma (10.3 deaths per million).² Systemic corticosteroids are the mainstay treatment for acute asthma exacerbation and status asthmaticus, and although they are lifesaving in these situations, we sought to further define their effect on the immune system of asthmatics. Chronic systemic corticosteroid use suppresses the immune response by reducing the activity and volume of the lymphatic system, resulting in lymphopenia. It is well established that there are subsequent decreases in immunoglobulins and complement levels following the chronic administration of systemic corticosteroids in patients with autoimmune dis-

orders.³ The quantified severity and the extent of immune suppression in asthmatic children receiving frequent systemic corticosteroid bursts have not been well reported in the literature.

Patients with poorly controlled, persistent asthma with frequent asthma exacerbations often receive multiple doses of systemic corticosteroids within a year. It is established that corticosteroids, especially in large doses, increase susceptibility to and mask symptoms of infection. Immunosuppression may result in activation of latent infection or exacerbation of intercurrent infections, and this could further exacerbate an individual's asthma. While the relationship between immunosuppression from chronic systemic corticosteroid administration and infection is well established,⁴ the risk of developing infections from frequent, periodic use of systemic corticosteroids in poorly controlled asthmatic children remains unclear.

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We conducted a retrospective chart review study to assess the extent and degree of immunosuppression by laboratory evaluation in children with poorly controlled, moderate-to-severe persistent asthma, who had received frequent systemic corticosteroid bursts (at least 2 times per year). These patients were seen in our asthma/allergy and immunology clinic due to frequent asthma exacerbations, many of which were precipitated by respiratory infections.

Materials and Methods

We conducted a descriptive case series study by means of retrospective chart review of 26 children, 3–15 years old with specialist-diagnosed, poorly controlled, moderate-to-severe persistent asthma. All patients were seen in the asthma/allergy and immunology clinic at a children's hospital. All patients received at least 2 systemic corticosteroid bursts within a year. The range of systemic corticosteroid bursts in our patients is between 2–20 times per year, with mean of 6.3 and median of 5 systemic corticosteroid bursts per year. The study's systemic corticosteroid burst regimen is prednisone 2 mg/kg (maximum 60 mg) for a loading dose, then 1 mg/kg/dose twice daily (maximum 30 mg/dose) to complete a 5 day course. All patients had immunology laboratory workup performed due to frequent respiratory tract infections (≥ 4 upper respiratory tract infections per year per parental report) and difficult to control asthma (need at least medium dose inhaled corticosteroid as a controller medicine and need at least 2 systemic corticosteroid bursts per year). All tests were completed outside the period of systemic corticosteroid burst at least 1 week apart (range: 1–12 weeks, mean: 4 weeks). Children with known primary or secondary immunodeficiency or those taking immunosuppressant agents other than corticosteroids were excluded from the study. The data collected include absolute T cell ($CD3^+$, $CD4^+$, and $CD8^+$), B cell ($CD19^+$), and natural killer (NK) cell ($CD3^- CD16^+ CD56^+$) counts, lymphocyte proliferation studies to phytohemagglutinin (PHA), concanavalin A (CON A) and pokeweed mitogen (PWM), immunoglobulin G, A, M, and antibody titers to tetanus, diphtheria, and pneumococcus (14 serotypes). The data for 26 patients were collected via an electronic medical records review and were compared against normal age-reference controls. This study was approved by local Institutional Review Boards (IRB).

All the laboratory tests were performed at a standardized commercial laboratory (ARUP[®] laboratories). Age appropriate reference intervals of all tests are standardized per ARUP laboratories. Absolute T, B, and NK cell counts were performed by quantitative flow cytometry using Lymphocyte Subset Panel 5—Total Lymphocyte Enumeration. Logical Observation Identifiers Names and Codes (LOINC) are 8122-4 ($CD3^+$ T cell counts), 24467-3 ($CD4^+$ T cell counts), 14135-8 ($CD8^+$ T cell counts), 15195-1 ($CD19^+$ B cell counts), and 20604-5 ($CD3^- CD16^+ CD56^+$ NK cell counts). Lymphocyte proliferation in response to the nonspecific mitogens PHA, CON A, and PWM (LOINC 58722-0) was determined by ³H-thymidine incorporation. Results are viewed as counts per minute (CPM) mitogen stimulated versus a control culture and a stimulation index, which represents the ratio of CPM of the stimulated lymphocytes to the mean CPM of the unstimulated control. Immunoglobulins G, A, and M were performed by quantitative nephelometry (LOINC 2465-3, 8, and 9). Tetanus and diphtheria IgG antibody titers (LOINC 53935-3 and 13227-4) were performed by quantitative mul-

tiplex bead assay. *Streptococcus pneumoniae* IgG antibodies (14 serotypes) were also performed by quantitative multiplex bead assay. These pneumococcal serotypes and LOINC are serotype 1 (LOINC 85954-6), 4 (86107-0), 5 (86130-2), 6B (27118-9), 3 (86080-9), 7F (25296-5), 9N (86169-0), 14 (85991-8), 8 (86147-6), 9V (30153-1), 12F (85977-7), 18C (27395-3), 19F (86024-7), and 23F (86064-3).

The data for T cell ($CD3^+$, $CD4^+$, and $CD8^+$), B cell ($CD19^+$), NK cell counts ($CD3^- CD16^+ CD56^+$), and immunoglobulins were collected, and frequency tables were generated that compared low values to normal age-reference controls per the standardized commercial laboratory (Supplementary Tables 1 and 2). The values for PHA-induced lymphocyte proliferation, CON A-induced lymphocyte proliferation, and PWM-induced lymphocyte proliferation were collected and frequency tables were done that compared the patient's values with normal controls. Frequency data were collected for lymphocyte proliferation that were <10 times the control (low lymphocyte proliferation), $10\text{--}20$ times the control (borderline low lymphocyte proliferation), and >20 times the control (normal lymphocyte proliferation). Frequency tables were generated for tetanus, diphtheria, and pneumococcal antibody titers (inadequate compared to normal control values). Antibody concentrations of >0.1 IU/mL are considered protective for diphtheria or tetanus. Antibody concentrations of ≥ 0.2 μ g/mL of at least 7/14 pneumococcal serotypes and ≥ 1.3 μ g/mL of at least 4/14 pneumococcal serotypes are considered protective for pneumococcal infections. Finally, crosstabs were done between $CD3^+$ T cells and PHA-induced lymphocyte proliferation, $CD4^+$ T cells and PHA-induced lymphocyte proliferation, $CD8^+$ T cells and PHA-induced lymphocyte proliferation, $CD3^+$ T cells and CON A-induced lymphocyte proliferation, $CD4^+$ T cells and CON A-induced lymphocyte proliferation, and $CD8^+$ T cells and CON A-induced lymphocyte proliferation. Analysis of variance (ANOVA) was used to investigate if any correlation exists between lymphocyte numbers and/or function, and different numbers of corticosteroid bursts. Pearson correlation coefficient was used to examine the data for an association between the timing of laboratory analysis and lymphocyte numbers/function. We used the software SPSS Statistics to analyze the data.

Results

This analysis comprised 26 asthmatic children. The cohort consisted of 15 males and 11 females. Patients' ages ranged from 3 to 15 years of age. The mean age was 7 years. Fifteen patients were African American, 7 were Caucasian, 3 were Hispanic, and 1 was Middle Eastern.

Twenty three patients had $CD4^+$ T cell and $CD8^+$ T cell counts drawn. The mean $CD4$ count was 1,371 cells/mm³, with a range of 98–2,782. Almost half of the subjects (47.8%, $n=11/23$) had low absolute $CD4^+$ T cell counts. The mean $CD8$ count was 700 cells/mm³, with a range of 91–1,453; only 8.7% ($n=2/23$) had low absolute $CD8^+$ T cell counts. Twenty four patients had absolute $CD3^+$ T cell counts drawn. The mean $CD3^+$ T cell counts was 2,184 cells/mm³, with a range of 160–4,486; 45.8% ($n=11/24$) had low $CD3^+$ T cell counts. Twenty four patients had absolute NK ($CD3^- CD56^+ CD16^+$) cell counts drawn and all were in the normal range (mean: 207, range: 61–1,200). None of the 24 patients had low absolute $CD19^+$ B cell count (mean: 697, range: 100–1,300). Table 1 demonstrates

TABLE 1. AGE, NUMBER OF STEROID BURSTS VERSUS IMMUNOLOGIC FINDINGS

Subject number	Age (year/gender/race)	Number of steroid bursts/year	CD3 ⁺ T cell counts (cells/mm ³)	CD4 ⁺ T cell counts (cells/mm ³)	CD8 ⁺ T cell counts (cells/mm ³)	CD19 ⁺ B cell counts (cell/mm ³)	NK cell counts (cell/mm ³)	Lymphocyte proliferation studies
1	3/male/black	2	2,700 (2,324–3,508)	1,368 (1,538–2,472)	972 (351–2,479)	648 (180–1,300)	144 (61–510)	PHA >20 × control CON A >20 × control PWM >20 × control
2	3/male/black	4	4,032 (2,324–3,508)	2,688 (1,538–2,472)	1,216 (351–2,479)	1,192 (180–1,300)	192 (61–510)	PHA <10 × control CON A <10 × control PWM >20 × control
3	3/female/black	4	1,620 (2,324–3,508)	828 (1,538–2,472)	432 (351–2,479)	880 (180–1,300)	170 (61–510)	PHA >20 × control CON A >20 × control PWM >20 × control
4	3/female/black	8	1,984 (2,324–3,508)	1,085 (1,538–2,472)	496 (351–2,479)	775 (180–1,300)	310 (61–510)	PHA <10 × control CON A 10–20 × control PWM >20 × control
5	3/male/black	10	1,667 (2,324–3,508)	1,110 (1,538–2,472)	486 (351–2,479)	685 (180–1,300)	324 (61–510)	Test was not available
6	3/male/Hispanic	20	1,890 (2,324–3,508)	1,050 (1,538–2,472)	480 (351–2,479)	722 (180–1,300)	358 (61–510)	Test was not available
7	4/male/white	5	1,652 (2,324–3,508)	1,366 (1,538–2,472)	850 (351–2,479)	646 (180–1,300)	102 (61–510)	PHA >20 × control CON A >20 × control PWM >20 × control
8	4/male/Hispanic	6	2,914 (2,324–3,508)	1,645 (1,538–2,472)	846 (351–2,479)	1,269 (180–1,300)	282 (61–510)	PHA <10 × control CON A <10 × control PWM 10–20 × control
9	5/female/Hispanic	3	4,486 (1,072–3,890)	2,782 (562–2,864)	1,453 (331–1,445)	1,189 (180–1,300)	448 (61–510)	PHA 10–20 × control CON A >20 × control PWM >20 × control
10	5/male/white	6	1,054 (1,072–3,890)	549 (562–2,864)	897 (351–2,479)	975 (180–1,300)	156 (61–510)	PHA 10–20 × control CON A >20 × control PWM >20 × control
11	6/male/black	2	2,583 (1,072–3,890)	1,640 (562–2,864)	861 (331–1,445)	761 (100–800)	369 (70–590)	PHA >20 × control CON A >20 × control PWM >20 × control
12	6/female/white	3	3,075 (1,072–3,890)	2,255 (562–2,864)	615 (331–1,445)	492 (100–800)	71 (70–590)	Test was not available
13	6/male/Middle Eastern	4	2,133 (1,072–3,890)	1,323 (562–2,864)	675 (331–1,445)	667 (100–800)	210 (70–590)	Test was not available
14	6/female/black	5	710 (1,072–3,890)	540 (562–2,864)	450 (351–2,479)	780 (100–800)	300 (70–590)	Test was not available

(continued)

TABLE 1. (CONTINUED)

Subject number	Age (year/gender/race)	Number of steroid bursts/year	CD3 ⁺ T cell counts (cells/mm ³)	CD4 ⁺ T cell counts (cells/mm ³)	CD8 ⁺ T cell counts (cells/mm ³)	CD19 ⁺ B cell counts (cell/mm ³)	NK cell counts (cell/mm ³)	Lymphocyte proliferation studies
15	7/male/white	3	210 (1,072–3,890)	98 (562–2,864)	91 (331–1,445)	406 (100–800)	81 (70–590)	PHA >20 × control CON A >20 × control PWM >20 × control
16	7/male/black	12	2,664 (1,072–3,890)	1,440 (562–2,864)	900 (331–1,445)	720 (100–800)	72 (70–590)	Test was not available
17	8/female/white	4	2,128 (1,072–3,890)	1,400 (562–2,864)	476 (331–1,445)	392 (100–800)	84 (70–590)	Test was not available
18	8/female/black	5	950 (1,072–3,890)	550 (562–2,864)	300 (331–1,445)	718 (100–800)	92 (70–590)	Test was not available
19	8/female/black	5	2,310 (1,072–3,890)	1,365 (562–2,864)	630 (331–1,445)	745 (100–800)	105 (70–590)	PHA >20 × control CON A >20 × control PWM >20 × control
20	8/female/white	7	Test was not available	Test was not available	Test was not available	Test was not available	Test was not available	PHA <10 × control CON A 10–20 × control PWM >20 × control
21	12/female/black	4	2,643 (1,072–3,890)	1,432 (562–2,864)	1,098 (331–1,445)	456 (120–740)	331 (92–1,200)	PHA 10–20 × control CON A >20 × control PWM >20 × control
22	12/female/black	8	2,775 (1,072–3,890)	1,739 (562–2,864)	814 (331–1,445)	703 (120–740)	111 (92–1,200)	PHA <10 × control CON A >20 × control PWM >20 × control
23	14/male/black	10	160 (1,072–3,890)	Test was not available	Test was not available	208 (120–740)	384 (92–1,200)	Test was not available
24	14/male/black	12	Test was not available	Test was not available	Test was not available	Test was not available	Test was not available	PHA >20 × control CON A >20 × control PWM >20 × control
25	15/male/white	5	832 (1,072–3,890)	455 (562–2,864)	351 (331–1,445)	288 (120–740)	108 (92–1,200)	PHA >20 × control CON A >20 × control PWM >20 × control
26	15/male/black	6	1,728 (1,072–3,890)	864 (562–2,864)	720 (331–1,445)	408 (120–740)	168 (92–1,200)	PHA <10 × control CON A >20 × control PWM >20 × control

The values represent the subject absolute lymphocyte counts. Normal age-reference controls per the standardized commercial laboratory are listed in parentheses. Bold means abnormal results. CON A, concanavalin A; NK, natural killer; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

TABLE 2. LYMPHOCYTE PROLIFERATION STUDIES

<i>Lymphocyte proliferation studies</i>	<i>PHA</i>	<i>CON A</i>	<i>PWM</i>
<10×control	35.3% (<i>n</i> =6/17)	11.8% (<i>n</i> =2/17)	0
10–20×control	17.6% (<i>n</i> =3/17)	11.8% (<i>n</i> =2/17)	5.9% (<i>n</i> =1/17)
>20×control	47.1% (<i>n</i> =8/17)	76.4% (<i>n</i> =13/17)	94.1% (<i>n</i> =16/17)
Total	17	17	17

age, number of steroid bursts per year, and immunologic findings.

Seventeen patients had lymphocyte proliferation studies performed, shown in Table 2. Of those who had PHA-induced lymphocyte proliferation studies, 35.3% (*n*=6/17) had low, 17.6% (*n*=3/17) had borderline low, and 47.1% (*n*=8/17) had normal lymphocyte proliferation. Of those who had CON A-induced lymphocyte proliferation studies, 11.8% (*n*=2/17) had low, 11.8% (*n*=2/17) had borderline low, and 76.4% (*n*=13/17) had normal lymphocyte proliferation. Of the patients who had PWM-induced lymphocyte proliferation studies, none had low, 5.9% (*n*=1/17) had borderline low, and 94.1% (*n*=16/17) had normal lymphocyte proliferation. Overall, negative correlations between T cell counts/lymphocyte functions versus the number of systemic corticosteroid bursts per year/timing of laboratory analysis were identified (Supplementary Tables S3–S5). However, none of these negative correlations was statistically significant except for the PHA-induced lymphocyte proliferation studies versus number of systemic corticosteroid bursts per year (Pearson's $r = -0.688$, $P = 0.003$).

All 26 patients had adequate levels of immunoglobulin G, A, and M per age reference and adequate protective antibody titers for tetanus (>0.1 IU/mL). All but one of the patients made adequate protective antibody titers for diphtheria (>0.1 IU/mL) and *S. pneumoniae* 14 serotypes (at least 4/14 serotypes ≥ 1.3 µg/mL and 7/14 serotypes ≥ 0.2 µg/mL). The immunoglobulins and antibody levels are shown in Supplementary Table S6.

The 1 patient with low antibody responses to *S. pneumoniae* had normal absolute CD3⁺, CD4⁺, and CD8⁺T cell counts. This patient is 5 years old, fully immunized, and received 3 systemic corticosteroid bursts per year. Her PHA-induced lymphocyte proliferation was borderline low, the CON A- and PWM-induced lymphocyte proliferations were normal. Her 14 serotypes pneumococcal antibodies were all ≥ 0.2 , but only 1 serotype was ≥ 1.3 . The patient's pneumo-

coccal antibody response after PCV23 immunization was adequate (4-fold rise in all serotypes).

These data were analyzed using contingency tables to examine the association between lymphocyte counts and lymphocyte proliferation studies. Fifteen patients in total had both T cell counts and lymphocyte proliferation studies available for crosstab analysis. The sample size was not large enough to look for a statistically significant effect.

The results for absolute CD4⁺ T cell counts compared to lymphocyte proliferation studies are shown in Table 3. Half of the subjects who had normal CD4⁺ T cell counts (*n*=4/8) demonstrated low PHA-induced lymphocyte proliferation, while 25% (*n*=2/8) showed borderline low PHA-induced lymphocyte proliferation. Twenty five percentage of the subjects who had normal CD4⁺ T cell counts (*n*=2/8) also demonstrated low CON A-induced lymphocyte proliferation. On the contrary, the majority of the subjects who had low CD4⁺ T cell counts demonstrated normal PHA-induced lymphocyte proliferation (71.4%, *n*=5/7), and Con-A-induced lymphocyte proliferation (85.7%, *n*=6/7).

The results for absolute CD8⁺ T cell counts compared to lymphocyte proliferation studies are shown in Tables 4. Of the patient who had normal CD8⁺ T cell counts (*n*=14), 35.7% (*n*=5/14) had low PHA-induced lymphocyte proliferation and 21.4% (*n*=3/14) had borderline low PHA-induced lymphocyte proliferation. To a lesser degree, these subjects also demonstrated low CON A-induced lymphocyte proliferation (14.3%, *n*=2/14) and borderline low CON A-induced lymphocyte proliferation (7.1%, *n*=1/14). In our cohort, the 1 patient who had low CD8⁺ T cell counts demonstrated normal PHA- and CON A- induced lymphocyte proliferation.

The results for absolute CD3⁺ T cell counts compared to lymphocyte proliferation studies are shown in Table 5. Of the patients who had normal CD3⁺ T cell counts (*n*=9), 44.5% (*n*=4/9) demonstrated low PHA-induced lymphocyte proliferation and 22.2% (*n*=2/9) had borderline low PHA-induced lymphocyte proliferation. Of the patients who had

TABLE 3. ABSOLUTE CD4⁺ T CELL COUNTS VERSUS LYMPHOCYTE PROLIFERATION STUDIES

		<i>PHA-induced lymphocyte proliferation studies</i>			
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD4 count	Low	14.3% (<i>n</i> =1/7)	14.3% (<i>n</i> =1/7)	71.4% (<i>n</i> =5/7)	7
	Normal	50% (<i>n</i> =4/8)	25% (<i>n</i> =2/8)	25% (<i>n</i> =2/8)	8
	Total	5	3	7	15
		<i>CON A-induced lymphocyte proliferation studies</i>			
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD4 count	Low	0	14.3% (<i>n</i> =1/7)	85.7% (<i>n</i> =6/7)	7
	Normal	25% (<i>n</i> =2/8)	0	75% (<i>n</i> =6/8)	8
	Total	2	1	12	15

TABLE 4. ABSOLUTE CD8⁺ T CELL COUNTS VERSUS LYMPHOCYTE PROLIFERATION STUDIES

<i>PHA-induced lymphocyte proliferation studies</i>					
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD8 count	Low	0	0	100% (<i>n</i> = 1/1)	1
	Normal	35.7% (<i>n</i> = 5/14)	21.4% (<i>n</i> = 3/14)	42.9% (<i>n</i> = 6/14)	14
	Total	5	3	7	15
<i>CON A-induced lymphocyte proliferation studies</i>					
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD8 count	Low	0	0	100% (<i>n</i> = 1/1)	1
	Normal	14.3% (<i>n</i> = 2/14)	7.1% (<i>n</i> = 1/14)	78.6% (<i>n</i> = 11/14)	14
	Total	1	0	14	15

normal CD3⁺T cell counts, 22.9% (*n* = 2/9) also showed low CON A-induced lymphocyte proliferation. On the contrary, the majority of the subjects who had low CD3⁺ T cell counts demonstrated normal PHA-induced lymphocyte proliferation (66.6%, *n* = 4/6) and CON A-induced lymphocyte proliferation (83.3%, *n* = 5/6).

Discussion

It is possible that susceptibility to viral respiratory infections is inherent in the asthmatic population, demonstrated by the childhood origins of asthma (COAST) study.⁵ However, the transient effect of immunosuppression by systemic corticosteroid use for asthma exacerbation/status asthmaticus could further increase the future risk of viral respiratory infections and subsequent asthma exacerbations. It would be difficult to elucidate whether recurrent asthma attacks triggered by viral infections are related to a weakened immune system (by systemic corticosteroid use) versus many other confounding factors known to predispose asthma exacerbations.

Approximately half of our patients had decreased numbers of absolute CD4⁺ and CD3⁺ T cell counts. In contrast, all the subjects had normal CD19⁺ B cell counts. Our results confirm the effect of systemic corticosteroids on T cell lymphopenia and that T cell subsets should potentially be tested more often in poorly controlled moderate-to-severe persistent asthma patients who frequently receive systemic corticosteroid.

The data from the lymphocyte proliferation studies exhibited variability. Many of the patients, although demon-

strating normal T cell counts, showed significant decrease in lymphocyte proliferation studies. No consistent relationship between lymphocyte counts and lymphocyte proliferation studies could be identified. The lack of correlation suggests that patients could be at risk for depressed lymphocyte function, even with normal counts.

The most consistent trend that emerged was that T lymphocyte number and function were decreased in a significant proportion of patients, while B lymphocyte number and function were normal. Further research is required, but these results indicate that systemic corticosteroid use in poorly controlled moderate-to-severe persistent asthma may be associated with a risk of depressed T lymphocyte count and function.

The role of T cells in asthma has been studied extensively. It is well established that Th2 cells are the classical cell type that promotes allergic inflammation and asthma. More recently, Th9 cells are reported to increase IgE production and promote the differentiation and proliferation of mast cells, which lead to the pathogenesis of asthma. Furthermore, Th17 cells have been shown to enhance Th2-mediated recruitment of eosinophils to the airways, neutrophilia in the airways, acute airway hyperresponsiveness, and mucus overproduction. Nevertheless, Treg cells prevent Th2-mediated inflammation of the airways in an IL-10-dependent way, while Th1-mediated immune responses are known for the adaptive immune response against several infections. However, our study has shown that systemic corticosteroid bursts used in asthma exacerbation can depress CD4⁺ helper T cell lymphocyte count and function; the specific subsets of Th cell

TABLE 5. ABSOLUTE CD3⁺ T CELL COUNTS VERSUS LYMPHOCYTE PROLIFERATION STUDIES

<i>PHA-induced lymphocyte proliferation studies</i>					
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD3 count	Low	16.7% (<i>n</i> = 1/6)	16.7% (<i>n</i> = 1/6)	66.6% (<i>n</i> = 4/6)	6
	Normal	44.5% (<i>n</i> = 4/9)	22.2% (<i>n</i> = 2/9)	33.3% (<i>n</i> = 3/9)	9
	Total	5	3	7	15
<i>CON A-induced lymphocyte proliferation studies</i>					
		<i><10×control</i>	<i>10–20×control</i>	<i>>20×control</i>	<i>Total</i>
CD3 count	Low	0	16.7% (<i>n</i> = 1/6)	83.3% (<i>n</i> = 5/6)	6
	Normal	22.2% (<i>n</i> = 2/9)	0	77.8% (<i>n</i> = 7/9)	9
	Total	2	1	12	15

have not been evaluated. With our data, we cannot estimate the extent and the degree of the T cell suppression in each of the helper T cell subsets.

There are many limitations in this pilot study, which lacks the sample size to fully calculate statistics. The data collection is limited due to the fact that subjects have a history of recurrent infections without pretreatment reference data, and the timing of posttreatment sampling is too variable to draw a firm conclusion. A follow-up investigation with a larger sample and control group, with immunologic studies repeated at different time points, would allow us to search for statistically significant relationships, assess the rate of recovery of lymphocyte levels/function after a systemic corticosteroid course, and to ascertain if there is a relationship between the presence of lymphopenia and the occurrence of infections.

The retrospective chart review we conducted provided valuable information but limited our ability to randomize the sample and reduce variability in the data. As this is an observational study using data from electronic medical records, the risk of bias and other sources of variability in the data are significant. All patients in this study resided in an inner-city environment, which may limit the extent to which the results can be applied to the general pediatric population.

This pilot study opens the door for future investigations and discussions. Data about frequency of infections and hospitalizations for each patient would broaden the scope and provide clinically relevant information. These data could be used to examine whether the *in vitro* changes in lymphocyte number and lymphocyte proliferations studies translate to clinically detectable outcomes.

A more complete understanding of the risks of systemic corticosteroids in asthmatics would improve clinical decision-making. While our sample size precludes complete calculation of statistics, the findings in this study can be clinically significant. Respiratory infections are recognized as the most important trigger of asthma in the pediatric population.⁶ Although bacterial infections, including sinusitis and pneumonia, have been reported as causes of asthma exacerbation, there is overwhelming evidence demonstrating the association between viral upper respiratory tract infections and asthma exacerbation.^{7–9} Prospective studies in asthma patients suggest that up to 85% of asthma exacerbations in children, and ~50% of similar episodes in adults, are caused by viral respiratory infections.^{10,11} Systemic corticosteroids are a universally used treatment for asthma exacerbation. If their use is associated with depressed immune function, then they also increase patients' risk for the most important trigger for exacerbation of asthma. This paradox represents a double-edged sword that physicians must wield with great care.

Treating asthma patients with systemic corticosteroids is unavoidable in certain situations, such as severe life-threatening asthma exacerbation. Expanding our understanding of the impact of systemic corticosteroids on our patients' immune systems is necessary, as these treatments may do more harm than good in some asthma patients or certain asthma phenotypes.

This investigation emphasizes the importance of adherence to asthma controller medications, and control of asthma triggers, to limit the number and dose of systemic corticosteroids given to patients. Immunizations are particularly important in pediatric patients with a history of frequent systemic corticosteroids. Future studies should evaluate the extent and clinical sequelae of the immunosuppressive effects of systemic corticosteroids in the context of treating asthma exacerbation.

Armed with this knowledge, clinicians will have a more refined understanding of optimal asthma treatment.

Author Disclosure Statement

No competing financial interests exist. All authors have nothing to disclose.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4
Supplementary Table S5
Supplementary Table S6

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