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Brief Communication

The Effect of Age on T-Regulatory Cell Number and Function in Patients With Asthma

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ABSTRACT

T-regulatory cells (Tregs) play a key role in suppressing effector cells and maintaining self-tolerance. Studies of younger adults and children suggest that insufficient differentiation and functional defects of Tregs may contribute to the development of asthma; however, data from older patients with asthma are limited. To address the effects of aging on the relationship of Treg frequency and function with clinical outcomes, we collected induced sputum (differential cell count and Treg frequency) and peripheral blood (Treg function and frequency) from aged (> 60 years of age) and younger (20–40 years old) patients with asthma. In younger patients, low Treg suppression was associated with significantly higher mean numbers of emergency department (ED) (1.8 vs. 0.17, $P = 0.02$) and urgent care visits (2.3 vs. 0.17, $P = 0.01$) for asthma, and decreased asthma control (mean Asthma Control Test [ACT] score, 17 vs. 21.3, $P = 0.01$) compared to those with high Treg suppression. In older patients, however, a lower Treg function was not significantly associated with ACT scores (18.2 vs. 13.4, $P = 0.10$), or the number of ED ($P = 0.9$) or urgent care visits ($P = 0.2$). Our data suggest that Tregs have a weak relationship with asthma control and clinical asthma outcomes in older patients and differ from findings in younger patients, where Tregs are more likely to play a protective role.

Keywords: Aging; T-lymphocytes, regulatory; cell count; asthma; self tolerance; sputum; blood; immunosenescence; cytokines; transcription factors

INTRODUCTION

Asthma is prevalent in older individuals with a reported incidence of 4% to 13% among adults > 65 years.[1] However, asthma is frequently underdiagnosed in this age group, making current statistics an underestimate. Moreover, rates are likely to increase with the aging of the United States population.[1] Most importantly, older adults have the highest rate of morbidity and mortality (> 5 times greater than younger groups).[2]

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A number of factors may contribute to poorer asthma outcomes in older adults including effects of aging on immune function. These age-related effects are complex, but fall into 2 pathways. "Immunosenescence" refers to a diminished or "blunted" response to pathogenic threats or tissue injury with aging. Immunosenescence is complicated by evidence that cells in the aged may not proliferate but remain alive, secreting low levels of inflammatory cytokines (*e.g.*, interleukin [IL]-1β, IL-6 and tumor necrosis factor [TNF]-α), a process termed "inflammaging."[3] Furthermore, the number and percentage of $CD4^+$ T regulatory cells (Tregs), as identified by the transcription factor, Foxp3, appear to increase with age.[4]

Although the contributions of Tregs in asthma are not fully defined, their number and function have been reported as lower in children and younger adults with asthma, suggesting a protective role.[5-7] However, Tregs have considerable functional diversity (*i.e.*, a variable capacity to suppress inflammation), which may alter clinical disease outcomes of asthma.[8] At present, knowledge is limited, with inconsistent results, on the effects of aging on Treg number and function in asthma.[9,10] Therefore, we sought to assess the effects of aging on the relationship of Treg frequency and function with asthma control and clinical outcomes.

MATERIALS AND METHODS

Patient population

Potential study participants were adults receiving medical care at a tertiary care center in New York City and were eligible if they were between the ages of 21–40 or ≥ 60 years, and had mild, moderate or severe persistent asthma, defined according to the National Heart, Lung and Blood Institute's Expert Panel on Asthma.[11] Study subjects who were potentially eligible were consecutively approached by the study physician (P.J.B.) during the weekly asthma and allergy outpatient clinic at the Icahn School of Medicine at Mount Sinai to determine their interest in participation. Those who were interested were scheduled to return for the initial study visit. Subjects were classified as having asthma, based upon meeting all of the following 3 criteria: 1) the presence of current episodic respiratory symptoms in the preceding 12 months, 2) a doctor's diagnosis of asthma and 3) evidence of either past or present reversible airflow obstruction (defined by the American Thoracic Society [ATS]/European Respiratory Society [ERS] guidelines).[12] Subjects with a current nonreversible obstruction, but a documented past reversibility were included. Individuals were excluded if they 1) had a diagnosis of chronic obstructive lung disease or emphysema, restrictive lung disease, or other chronic respiratory illnesses, 2) had a smoking history of ≥ 10 pack-years, 3) had dementia, 4) received immunosuppressive medications (excluding corticosteroids) in the past 12 months or 5) were pregnant or lactating. To limit confounding due to co-morbidities, we excluded subjects that did not meet eligibility criteria for immune-gerontological studies.[13] These criteria included exclusions based upon the following characteristics which could alter immune function including: clinical history (*i.e.*, lymphoproliferative disorder, collagen-vascular disease, inflammatory bowel disease) and severely abnormal recent laboratory data (*i.e.*, white blood cells < $2.0 \times 10^3/cm^3$, platelets < 75/μL). To adjust for potential age-related effects on airway inflammation, we recruited age-matched controls who met the same inclusion and exclusion criteria, but did not have a history of past or current asthma. Each subject provided written informed consent. The study was approved by The Human Research Protection Program at the Icahn School of Medicine at Mount Sinai.

Clinical data and inflammatory outcomes were collected over 3 in-person visits. On the first visit, sociodemographic characteristics, asthma history, asthma morbidity over the 12 months

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prior to study enrollment, current asthma medications, Charlson Comorbidity Index,[14] and smoking history were obtained. To access for medication adherence, we administered the Medication Adherence Report Scale for Asthma (MARS-A), a validated tool to measure adherence with inhaled corticosteroids among patients with persistent asthma. The MARS-A is a 10-item questionnaire which assesses both intentional and nonintentional nonadherence, in which medication use is rated on a 5-point Likert scale (1 indicating always, to 5 indicating never).[15] Subjects were classified as atopic if they had a detectable serum IgE level (> 0.35 kIU/mL) or a positive skin test reaction (>3-mm wheal, and a positive histamine and negative saline controls) to at least one of the following antigens: house dust mites (*Dermatophagoides farinae, Dermatophagoides pteronyssinus*), cockroach (*Blattella germanica*), mixed molds (*Alternaria, Aspergillus, Penicillinase* and *Sphaerospemum*), cat, dog, tree mix, grass mix, ragweed or weed mix.

Sputum induction and processing

Subjects were scheduled to return for sputum induction 2 weeks after the initial intake visit. They were excluded from sputum induction if they reported 1) an upper respiratory tract infection, fever or asthma exacerbation defined as worsening of asthma symptoms requiring prescription of systemic corticosteroids[16] 2) surgery within the past 4 weeks in order to exclude alternation of inflammation due to alternative etiologies besides asthma. Prior to sputum induction, subjects underwent spirometry. After treatment with 360 μg of albuterol, spirometry was repeated to access for airway reversibility, and then sputum was induced by nebulized hypertonic saline (3%) as previously described.[17] Sputum was processed by the whole sputum method, adopting the protocol used by SARP.[18] A portion of the sputum samples was weighed, a dithiothreitol (DTT) containing Sputolysin Reagent (Calbiochem, San Diego, CA, USA) added (1 mL to 1 g of sample), placed in a 37°C shaker for 15 minutes, and then washed with phosphate-buffered saline (PBS). Cytospins were prepared and stained with Diff-Quick (Dade Diagnostics of PR, Aguada, Puerto Rico) for differential cell counts and read as previously described.[17,19]

Measurement of sputum Tregs

We measured Tregs from the sputum by flow cytometry, adapting previously published protocols.[20-22] A sample of the sputum cell pellet was re-suspended in flow cytometry staining (FACS) buffer (PBS + 1% bovine serum albumin + 2 mM ethylenediaminetetraacetic acid) with 5% mouse serum and transferred to FACS tubes. Cellular staining was performed using the antibodies of interest, at 4°C for 30 minutes. Treg subsets were examined using monoclonal antibodies to CD4 (Pacific Blue), CD3 (PerCP-Cy5) and CD127 (PE), followed by intracellular staining for Foxp3 (FITC) after fixing/permeabilizing cells overnight in Fixation/Permeabilization Working Solution from Foxp3 Staining Buffer Set. All antibodies were purchased from eBioscience (San Diego, CA, USA). Samples were measured using an LSR II Flow Cytometer (BD Biosciences, San Jose, CA, USA) and data analysis using FlowJo software (Tree Star, Inc., Ashland, OR, USA). Tregs were marked by $CD3^+CD4^+$ cells expressing Foxp3 and $CD127^{low}$.[22] The frequency of true positive cells was determined by subtracting the value of isotype control from the value of the sample.

Treg isolation and enumeration

Subjects returned for the third visit (within 24 hours of sputum induction) to collect peripheral blood mononuclear cells (PBMCs) and asthma control (as assessed by the Asthma Control Test [ACT]). PBMCs were isolated from peripheral blood using Ficoll-Paque (GE Lifesciences, Marlborough, MA, USA) gradient centrifugation. Tregs were isolated and purified from PBMCs using EasySep Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation

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Kit (Stemcell Technologies, Vancouver, BC, Canada) according to the manufacture's instructions. Isolated cells were stained using the antibodies of interest (Invitrogen, Carlsbad, CA, USA) at 4°C for 30 minutes. Treg subsets were examined using monoclonal antibodies to CD4 (Pacific Blue), CD3 (PerCP-Cy5) and CD127 (PE), followed by intracellular staining for Foxp3 (FITC) after fixing/permeabilizing cells overnight in BD Cytofix/Cytoperm (BD Bioscience). The samples were acquired on a BD LSR II flow cytometer and the analysis was performed using FlowJo software (FlowJo LLC, Ashland, OR, USA). Tregs were reported as the percentage of $CD3^+CD4^+$ cells expressing $Foxp3^+$, $CD127^{low}$.

Coculture suppression assay

The function of peripheral Tregs was measured by its ability to suppress autologous T-cell proliferation in culture with carboxyfluorescein succinimidyl ester (CFSE) dilution. Isolated cells were cultured in RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum + 2% penicillin streptomycin in 96-well plates. PBMCs (100,000 cells/0.1 mL) were cocultured with $CD4^+CD127^{low}$ Tregs (at decreasing ratios of PBMC:Tregs, from 1:1 to 1:32) for 72 hours in the presence of anti-CD3/anti-CD28 activation beads (Dynabeads™; Gibco BRL, Gaithersburg, MD, USA). For each sample, CFSE dilution was evaluated in different concentrations of CD^+CD127^{low} cells. For each sample and co-culture condition, controls were performed in the absence of stimulus, revealing absent or negligible cellular proliferation. The samples were acquired and analyzed as above. The percent suppression at each dilution was plotted for each subject and the area under the curve (AUC) was calculated using the trapezoid rule.

As Treg function is diverse even within a defined cellular subset, which can alter clinical outcomes of disease (*i.e.*, cancer and rheumatoid arthritis), we classified patients into high and low Treg functioning groups.[23,24] The definition of high versus low Treg suppression was based upon suppression levels above or below, respectively, the mean AUC for all subjects (asthma and control) of 18.9. The decision to define high and low Treg suppressor based upon levels above or below, respectively, a mean outcome value was based upon the definition of T2 high and low asthma.[25]

Statistical analysis

Statistical analyses were performed with R (R version 3.5.0 [2018-04-23]. Categorical data were analyzed using the χ^2 test. Differences between groups for normally distributed variables were computed by means of analysis of variance (ANOVA) and its pairwise differences, while Kruskal-Wallis non-parametric ANOVA was employed for non-normal variables. There was no correction for multiple comparisons.

RESULTS

Sixteen aged (A; mean age 66.8 ± 4.1 years) and 16 younger (Y; mean age 31.3 ± 5.6 years) patients with asthma were evaluated. Subjects were further grouped based upon their peripheral Treg function (high [H] or low [L] suppressor) for a total of 4 groups: aged low-Treg (AL), aged high-Treg (AH), younger low-Treg (YL) and younger high-Treg (YH) function (**Table 1**). These groups of subjects with asthma were chosen for the following 2 reasons:1) one is that the underlying goal of this study was to examine Treg function with age and asthma outcomes and 2) the other is that that there is significant diversity of Treg function. In addition, 14 aged (mean age 68.5 ± 5.3 years) and 15 younger (mean age 26.7 ± 4.4 years) controls were recruited.

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Table 1. Baseline characteristics of participants according to age and Treg function

Characteristics	Asthma				*P* value		
	AL (n = 8)	AH (n = 8)	YL (n = 10)	YH (n = 6)	Overall	AL vs. AH	YL vs. YH
Demographics							
Age	68.1 ± 3.98	65.5 ± 4.11	33 ± 5.73	25.8 ± 4.32	< **0.01**	0.22	0.10
Female	6 (75.0)	4 (50.0)	7 (70.0)	4 (66.7)	0.79	0.61	1
Atopy	5 (62.5)	7 (87.5)	10 (100)	6 (100)	0.09	0.57	
Comorbidity Index score	4.13 (2.59)	3 (1.07)	0.20 (0.42)	0 (0.00)	< **0.01**	0.28	0.17
Smoking status							
Never smoked	5 (62.5)	4 (50.0)	8 (80.0)	5 (83.3)	0.49	1	1
Past smoker	3 (37.5)	4 (50.0)	2 (20.0)	1 (16.7)	0.49	1	1
Pack years	3.68 ± 5.06	6.38 ± 2.79	1.80 ± 1.13	0	0.30	0.59	
Asthma history							
Age of asthma onset	36.8 ± 18.6	26.7 ± 27.1	4.60 ± 4.27	5.17 ± 2.32	< **0.01**	0.43	0.74
Years with asthma	32.5 ± 17.2	38.9 ± 23.7	28.5 ± 7.79	22.5 ± 4.18	0.27	0.57	0.07
Positive family history of asthma	4 (50.0)	5 (62.5)	8 (80.0)	5 (83.3)	0.36	1	1
Spirometry (FEV1/FVC)	89.9 ± 8.72	83.0 ± 10.6	91.1 ± 10.5	89.5 ± 8.87	0.35	0.18	0.75
Peripheral Treg function							
AUC	4.3 ± 4.9	39.5 ± 19.7	4.1 ± 3.7	60.6 ± 16.8	< **0.01**	< **0.01**	< **0.01**
Sputum differential (%)							
Macrophages	58.6 (43.1–80.1)	77.9 (73.3–82.9)	95.8 (90.0–97.2)	88.5 (87.7–91.1)	**0.03**	0.22	0.36
Neutrophils	24.1 (13.0–41.7)	17.3 (13.9–21.0)	2.51 (1.49–8.12)	6.93 (6.84–9.02)	**0.04**	0.34	0.36
Eosinophils	9.79 (2.04–13.3)	2.72 (2.15–3.59)	1.02 (0.16–1.93)	1.18 (0.99–1.64)	0.05	0.28	0.58
Lymphocytes	0.57 (0.53–0.77)	0.50 (0.45–0.90)	0.58 (0.48–0.71)	0.61 (0.53–0.82)	0.93	0.56	0.71
Treg frequency							
Sputum Tregs, % $Foxp3^+$, $CD127^{low}$	17.8 (15.9–18.9)	5.70 (5.09–7.49)	8.06 (5.75–15.9)	6.45 (5.16–7.20)	0.36	0.12	0.56
Peripheral Tregs, % $Foxp3^+$, $CD127^{low}$	5.06 (3.16–8.10)	7.55 (5.55–13.9)	5.29 (3.37–8.22)	15.6 (10.6–28.9)	0.55	0.63	0.14

Data are presented in absolute numbers (%), mean ± standard deviation or median (interquartile range). Bold-faced values are significant.
AL, aged low-T regulatory cell; AH, aged high-T regulatory cell; YL, younger low-T regulatory cell; YH, younger high-ET regulatory cell; Treg, T regulatory cell; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; AUC, area under curve.

Overall, there were no significant differences in duration of asthma, allergic sensitization, lung function, dose of inhaled corticosteroids (data not shown) or smoking history among the 4 groups of patients with asthma (**Table 1**). We did not observe any difference between our asthma subjects on their medication adherence report scale (MARS-A, $P = 0.93$) and therefore did not consider it necessary to adjust in the analysis. Aged (AH, AL) patients with asthma had significantly larger numbers of sputum neutrophils and eosinophils compared to younger patients (Y.H. and Y.L.). We did not find any significant differences between our 4 groups of patients with asthma in regards to the frequency of peripheral and sputum Tregs (**Table 1**). Furthermore, we did not observe any association between the frequencies of peripheral and sputum Tregs for any of the asthmatic and control subjects (data not shown).

Asthma outcomes differed significantly, depending upon age and Treg function (**Table 2**). For example, in younger patients with asthma, those with low compared to high Treg

Table 2. Asthma outcomes according to age and Treg function

Asthma outcomes	Asthma				*P* value	
	AL (n = 8)	AH (n = 8)	YL (n = 10)	YH (n = 6)	AL vs. AH	YL vs. YH
ACT score	18.2 ± 6.36	13.4 ± 4.24	17.0 ± 3.46	21.3 ± 1.86	0.10	**0.01**
History of intubation ever	0 (0.00)	2.00 (28.6)	1 (10.0)	1 (16.7)	0.20	1
Outcomes in past 12 months:						
No. ER visits/patient	1.12 ± 2.47	1 ± 1.29	1.80 ± 1.75	0.17 ± 0.41	0.90	**0.02**
No. Hospitalizations/patient	0.50 ± 1.41	0.29 ± 0.76	0.40 ± 0.70	0 ± 0.00	0.72	0.10
No. Urgent care visits/patient	0.88 ± 1.46	3 ± 3.79	2.30 ± 1.95	0.17 ± 0.41	0.20	**0.01**
No. Patients on oral/IV CS	5 (62.5)	5 (71.4)	9 (90.0)	3 (50.0)	1	0.12

Data are presented in absolute numbers (%) or mean ± standard deviation. Bold-faced values are significant.
Treg, T regulatory cell; AL, aged low-T regulatory cell; AH, aged high-T regulatory cell; YL, younger low-T regulatory cell; YH, younger high-ET regulatory cell; ACT, Asthma Control Test; ER, emergency room; CS, corticosteroids; IV, intravenous.

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Table 3. Treg frequencies and peripheral function of subjects with asthma and controls

Inflammatory outcomes	AsA (n = 16)	AsY (n = 16)	CA (n = 14)	CY (n = 15)	*P* value			
					AsA vs. AsY	AsA vs. CA	CA vs. CY	AsY vs. CY
Sputum Tregs, % Foxp3$^+$, CD127^{low}	7.49 (5.51–18.1)	7.20 (5.16–14.4)	13.7 (7.69–16.6)	18.3 (15.4–24.0)	0.79	0.69	0.06	**0.03**
Peripheral Tregs, % Foxp3$^+$, CD127^{low}	6.41 (3.97–10.6)	6.92 (4.94–13.8)	7.81 (3.3–16.8)	5.25 (3.03–10.5)	0.95	0.74	0.62	0.66
Peripheral Treg function, AUC								
AUC	21.9 ± 22.3	25.3 ± 30.0	8.5 ± 7.7	17.4 ± 16.1	0.72	**0.04**	0.07	0.37

Data are presented in means ± standard deviation or median (interquartile range). Bold-faced values are significant.
AsA, aged subjects with asthma; AsY, younger subjects with asthma; CA, aged subject in control; CY, younger subjects in control; AUC, area under curve.

suppression had significantly larger number of ED (1.8 vs. 0.17, $P = 0.02$) and urgent care visits (2.3 vs. 0.17, $P = 0.01$) for asthma, and decreased current asthma control (mean ACT score, 17 vs. 21.3, $P = 0.01$). These differences in outcomes between the 2 groups of younger patients with asthma (YL vs. YH) were not attributable to differences in frequency of sputum neutrophils (2.51 vs. 6.93 respectively, $P = 0.36$) or eosinophils (1.02 vs. 1.18, $P = 0.58$; **Table 1**). Conversely, in aged patients, Treg function had less impact on asthma outcomes. In the aged group, a lower compared to a higher Treg function was not significantly associated with ACT scores (18.2 vs. 13.4, $P = 0.10$) or the numbers of ED ($P = 0.9$) or urgent care visits ($P = 0.2$) in the past 12 months. Our data suggest that younger asthma patients with high vs. low Treg function have lower morbidity; in contrast, higher Treg function was not associated with these outcomes in older patients.

Similar to other studies, we also found that in younger subjects, the numbers of sputum and peripheral Tregs were smaller in patients with asthma compared to controls (7.20 vs. 18.3, $P = 0.03$, **Table 3**). Conversely, in aged subjects, those with asthma compared to controls showed no significant difference in the frequency of sputum (7.49 vs. 13.7, $P = 0.69$) or peripheral Tregs (6.41 vs. 7.81, $P = 0.74$), further suggesting a potential limited role of Tregs in older asthmatic patients.

DISCUSSION

Older patients with asthma experience increased morbidity and mortality compared to younger patients.[1,2] Although coexisting lung disease, comorbidities as well as underdiagnosis and undertreatment of asthma contribute to these poorer outcomes, the effect of aging on inflammation is also a contributing factor. We have previously demonstrated that differences in airway inflammation, specifically increased numbers of neutrophils and eosinophils, and expression of cytokines (*i.e.*, IL-6 and IL-8) is associated with decreased asthma control in older patients with asthma.[17] In the present study, we further evaluated the association of clinical outcomes of asthma in aged patients with Treg number and function. Our data suggest that Tregs may have a weak relationship with clinical asthma outcomes in older patients, and are unlike findings in younger patients where Tregs are more likely to have a protective role.[6,26,27]

Tregs, which are originally characterized by CD4$^+$CD25$^+$ and later with the identification of Foxp3—a forkhead family transcription factor—as a critical molecule in its development, have multiple functions to suppress inflammation and promote tolerance.[7] Improving Treg function through inhaled corticosteroids[6] or through allergen immunotherapy[28] may be a potential mechanism to improve asthma outcomes. The effect of aging and the role of Tregs in asthma is limited. However, it is has been well documented that with aging, there is a

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loss of protective functions of Tregs (despite an increase in their number), which has been suggested to play a role in a decreased control of autoimmune inflammation, and immune hypo-responsiveness to infection, vaccination and tumors.[4] Therefore, we hypothesized that in older patients with asthma, Treg function would be decreased due to aging and asthma, and may contribute to poorer outcomes in this age group.

Two prior studies have examined the number of Tregs in peripheral blood of older patients with asthma, with conflicting results. For example, the percentage of peripheral Tregs, identified by $CD3^{+}CD4^{+}CD25^{high}CD127^{low}$, in older patients with asthma (mean age 72 years, n = 95) was not significantly different from age-matched controls, irrespective of disease severity.[9] We also found that the number of peripheral Tregs was not significantly different in the older subjects with asthma compared to the age-matched controls. However, in another published study, PBMCs from older patients with asthma (mean age 72 years, n = 32), when stimulated with anti-CD3/anti-CD28, had significantly decreased Foxp3 mRNA expression compared to age-matched controls (n = 17).[10] Unlike the previous studies, we also examined if there were significant differences in sputum Treg frequencies between aged subjects with asthma and controls. Similar to our findings in the peripheral blood, we did not detect a significant difference between the 2 groups. Additionally, we looked at whether the suppressive function of peripheral Tregs differed between older subjects with and without asthma, and found that Treg function was higher in the former group. Furthermore, unlike previous studies, we evaluated the potential role of Treg function with current asthma control and asthma resource utilization in the past 12 months.

To our knowledge, this is the first study to compare Treg frequencies and function with clinical outcomes between a younger and an older adult population of asthmatic patients. Neither of the above-mentioned studies in aged patients had a younger comparison group. Overall, we did not find any significant differences between the frequencies of Tregs in the peripheral blood or sputum between older and younger patients with asthma. Similar to previous studies in children with asthma, we also found a significantly lower number of Tregs in the airway samples from younger patients compared to their age-matched controls.[6] Although there was a trend towards fewer numbers of sputum Tregs in aged patients with asthma compared to their age-matched controls, it was not significant. We did not find significant differences in the peripheral Treg frequencies between any of the groups of subjects we studied. Of note, there was no significant correlation between the peripheral and sputum Treg frequencies, as has previously been demonstrated[20] in younger adults with asthma.

Our next question was to begin to address whether the Treg function may contribute to differences in clinical outcomes. Our data suggest that in aged subjects with asthma, the Treg suppressive function may not significantly impact current asthma control as well as a previous 12-month history of hospitalizations, urgent and ED visits, or use of corticosteroid bursts. This is in contrast to the younger population in which a lower Treg function was related to poorer asthma control and morbidity markers in the prior 12 months. There may be a potential explanation for increased asthma resource utilizations in the past 12 months in older patients with asthma and higher Treg function. While higher Treg suppressive function may limit airway inflammation in asthma, it may also compromise host defense immunity against microorganisms,[29] often exacerbating asthma and leading to increased ED visits or hospitalizations. We also acknowledge that the suppressive capabilities and frequencies of Tregs likely vary over time points. In our report, this is less likely of an impact on asthma control, as ACT was collected at the time of suppression assays, but may have potentially

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impacted on the past 12 months of asthma resource utilization. We also acknowledge that our study was also not performed prospectively, and that controller medications or medication adherence may have differed during this time. However, based upon the MARS-A, we did not observe any significant differences between asthma groups in controller medication adherence.

In conclusion, our study suggests that in older patients with asthma, Tregs may play a limited role in clinical outcomes. However, additional studies with larger sample sizes are warranted to further evaluate the role of Tregs in this at-risk group of patients. If validated in larger studies, these findings suggest that other age-related inflammatory changes may account for and should be explored as the etiology of poorer outcomes in some aged patients with asthma.

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