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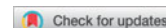
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The number and function of T regulatory cells in obese atopic female asthmatics

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ABSTRACT

Background: Mechanisms underlying the association between asthma and obesity remain poorly understood. Obesity appears to be a risk factor for asthma, and obese asthmatics fare poorly compared to lean asthmatics. **Objectives:** To explore the possibility that reduced regulatory T cell (Treg) number and function contribute to the obesity-asthma association. We concentrated on obese females with childhood-onset asthma, since Treg may be involved in this phenotype. **Methods:** We recruited 64 women (ages 18–50) into four groups: lean (BMI 18–25 kg/m²) controls ($n = 17$) and asthmatics ($n = 13$), and obese (BMI ≥ 35 kg/m²) controls ($n = 17$) and asthmatics ($n = 17$). Asthmatics had atopy and childhood-diagnosed asthma. We assessed lung function, asthma control and quality of life. Peripheral blood CD4⁺/CD25⁺/FoxP3⁺ Treg cells were identified and counted by flow cytometry and expressed as % total CD4⁺ T cells. We assessed Treg cell function by the ability of CD4⁺/CD25⁺ Treg cells to suppress autologous CD4⁺/CD25⁺ responder T cell (Tresp) proliferation and measured as % suppression of Tresp cell proliferation. **Results:** Obese asthmatics had worse lung function, asthma control, and quality of life compared to lean asthmatics. Compared to lean or obese control groups, the number of Treg cells in the obese asthmatics was approximately 1.58- or 1.73-fold higher. The ability of Treg cells from obese-asthmatics to suppress Tresp cell proliferation was reduced. **Conclusions:** Obese, atopic women with childhood diagnosed asthma demonstrate increased Treg cell number and mildly decreased Treg cell function. Our data do not support the view that reduced Treg cell number contributes to this obese-asthma phenotype.

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Atopy; early onset asthma; obesity; phenotype; T cells

Introduction

Asthma and obesity are common chronic diseases affecting millions. Prospective cohort studies and meta-analyses had uncovered a close association between obesity and asthma (1,2). Not only is obesity recognized as a significant risk factor for the development of asthma; obese individuals have a higher incidence and prevalence of asthma, compared with healthy weight individuals. Additionally, obesity has a significant impact on treatment and prognosis of asthma. Obesity is associated with poorer asthma control, increased limitation in the performance of daily activities and inferior quality of life (1–4).

Prior studies attempted to clarify whether the immune dysregulation found in asthma is compounded by obesity. Such studies have arrived at different conclusions. Human studies were not definitive, and neither were studies using mouse models of asthma (reviewed in (5)). Furthermore, several researchers found no relationship between obesity-associated asthma and markers of inflammation (5). Others have attributed airway hyperreactivity (AHR)

in the obese asthmatic to obesity-related physiological changes (6).

Differences in the conclusions from prior studies were attributed to age, sex, the degree of obesity and the strain of mice (5,7). More recent data suggests the presence of several phenotypes of asthma associated with obesity. Indeed, current knowledge divides the obese-asthma phenotype into at least two distinct subsets (5,8–10). Those with later onset asthma accompanied by fewer markers of allergic inflammation and a lesser amount of airway reactivity and whose asthma improves with weight loss. This adult-onset asthma-obesity phenotype is likely to be a consequence of obesity-induced metabolic changes and appears to be driven by distinct processes including neutrophils or paucigranulocytic cells (5,10). Another phenotype, examined in this study, is characterized by an earlier-onset of asthma and elevated markers of inflammation in a T helper type 2 (Th2)-dependent fashion. These obese asthmatics are also more likely to be atopic. In this subset, asthma is thought to be potentiated by the

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obesity. We concentrate on this obese phenotype of childhood-onset asthma with atopy. The prevailing paradigm in this subset is that Th2 lymphocytes drive inflammation (3).

While the exact mechanisms remain poorly understood (3–7), there is persuasive evidence supporting an essential role of a subset of T cells; the regulatory T cells (Treg) (11,12). Treg cells inhibit Th2 cell proliferation, reduce allergen-specific IgE secretion, suppress effector T-cell migration to tissues and activity, limit eosinophils, mast cells and basophils interaction with resident tissue cells, suppress airway inflammation and hyperresponsiveness, and decrease mucous hypersecretion and airway remodeling (10,13,14). Supporting this hypothesis are data showing that asthmatics have fewer and less functional Treg cells, compared to non-asthmatics (15). Likewise, obese individuals without asthma have a reduced number of Treg cells, compared to their lean counterparts (16).

It is then fitting to assume that either Treg insufficiency or aberrant Treg function represent an important cellular mechanism linking obesity and asthma. However, the role of Treg cells in the obesity-asthma relationship remains mostly unexplored, and studies have again demonstrated different results (15,17). Prior investigations did not differentiate between the phenotypes of obese asthmatics and included obese asthmatics of different ages and sex (5,7). To our knowledge, there has been one study that examined Treg cell number in obese children with asthma, but this study did not assess Treg function (18). In this study, we compared both the number and function of Treg cells between lean and obese asthmatics who were atopic and whose asthma was diagnosed in childhood. We studied only women as the relationship between obesity and asthma is most active in adult females (2,7).

Methods

Subjects

The institutional review board approved this study (11-076B). Informed written consent was obtained from all participants.

Sixty-four females between the ages of 18 and 50 years were recruited into one of the following four groups: normal-weight non-asthma controls (Nw-C, $n = 17$), obese non-asthma controls (Ob-C, $n = 17$), normal-weight atopic asthma (Nw-A, $n = 13$) and obese atopic asthma (Ob-A, $n = 17$). We defined obesity as a body mass index (BMI) ≥ 35 kg/m² and lean as a BMI between 18 and 25 kg/m². All asthmatics had a prior physician diagnosis of asthma before age 12. We defined atopy as a history of allergy. We defined asthma as typical symptoms

with either of the following two requirements. An increase in FEV1 of 12% or more following bronchodilator administration; or a significant airway hyper-responsiveness (AHR), defined as a provocative dose of inhaled methacholine causing a 20% reduction in FEV1 (PD₂₀) of <8 mmol. A respiratory therapist performed spirometry (Sensormedics Vmax series, Yorba Linda, CA) and results were expressed as a percentage of predicted. We minimized potential confounders by recruiting only premenopausal women since the association between obesity and asthma is different in postmenopausal women (2,7). Exclusion criteria included: current smoker or ex-smoker with >10 pack-year smoking history, any comorbidity that could potentially increase systemic inflammation such as an active infection, the presence of diabetes and recent medication use that could alter systemic inflammation. We excluded subjects who took of oral steroids, statins or hormonal contraceptives within four weeks of enrollment; We also eliminated subjects with any acute respiratory symptoms, history of obstructive sleep apnea (OSA), hepatic and cardiac disease, or AHR in the non-asthmatic subjects. We used the STOP-Bang questionnaire to screen for OSA in obese subjects (19). Those with scores of ≥ 3 were excluded. The adequacy of asthma control and asthma disease-specific health-related quality of life were assessed using the Asthma Control Questionnaire (ACQ) and the Asthma Quality of Life Questionnaire (AQLQ).

Treg Isolation and purification

Heparinized blood was obtained from all subjects following informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque™ Plus (GE Life Sciences, Pittsburgh, PA) density-gradient centrifugation. The PBMCs were divided into two parts; part one was used for the identification and enumeration of CD4⁺/CD25⁺/FoxP3⁺ Treg cells by flow cytometry. The remaining cells were used for the isolation of CD4⁺/CD25⁺ Treg cells and CD4⁺/CD25⁻ responder T cells (Tresp) using human regulatory T cell isolation kit (Miltenyi Biotec, Sunnyvale, CA) following the manufacturer's protocol.

Fluorescence-activated cell sorting (FACS)

CD4⁺/CD25⁺/FoxP3⁺ Treg cells were identified and quantified using human Treg Detection kit (Miltenyi Biotec, Sunnyvale, CA) and FACS analysis. PBMCs were stained with fluorescein isothiocyanate-conjugated anti-CD4 and allophycocyanin-conjugated anti-CD25 antibodies. After washing, the cells were fixed and permeabilized in fixation/permeabilization solution,

blocked with FcR blocking reagent and stained with phycoerythrin-conjugated anti-FoxP3 antibody. Fluorescence minus one controls were stained in parallel. FACS data acquisition and sorting were conducted using BD FACSVerse flow cytometer (BD Biosciences, San Jose, CA). Raw data were processed with FlowJo software (Treestar, Ashland, OR). PBMCs were gated on CD4+ lymphocytes based on forward, and side light scatter and CD4 staining. The CD4+/CD25+/FoxP3+ Treg subpopulation within the T lymphocytes population was identified and counted. The number of Treg cells was expressed as a percentage of CD4+ T cells for each subject and compared among the four groups.

Treg suppression assay

Treg cell function was tested by measuring their ability to suppress autologous Tresp cell proliferation using human Treg Suppression Inspector Kit following the manufacturer's protocol (Miltenyi Biotec, Sunnyvale, CA). In brief, 5×10^4 CD4+/CD25- Tresp cells were cultured alone or were co-cultured with autologous CD4+/CD25+ Treg cells at a Treg-to-Tresp cell ratio of 1:4, 1:2 or 1:1 in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were stimulated with MACSibead particles pre-loaded with CD2, CD3, and CD28 antibody to induce Tresp cell proliferation and expansion, and incubated at 37 °C and 5% CO₂ in a humidified incubator for five days. One μ Ci of ³H-thymidine was added to each well for the last 16 hours of incubation. Cells were washed, harvested and ³H-thymidine incorporation measured using a liquid scintillation counter. Cell proliferation was assessed by ³H-thymidine incorporation. Suppression of Tresp cell proliferation by Treg cells was expressed as a percentage inhibition of Tresp cell proliferation.

Serum IgE measurement

Serum was collected at the time of study enrollment. Serum IgE was subsequently quantified using the Phadia

ImmunoCAP Total IgE assay (Phadia US, Portage, Michigan). We defined an IgE level < 100 IU/ml as normal.

Statistical analysis

We powered the study to detect a difference in Treg cells between asthmatics and nonasthmatics. We estimated the number of Treg cells from prior studies (15–18,20,21). Assuming a Treg level of 0.025 cells/mL $\times 10^6$ (standard deviation 0.1) in obese control individuals and 0.04 cells/mL $\times 10^6$ in obese asthmatics. Recruiting 11 subjects in each group would yield an 80% power to detect a significant difference with a two-sided α of 0.05.

The number of Treg cells was expressed as a percentage of total CD4+ T cells, and suppression of Tresp cell proliferation by Treg cells was expressed as a percentage inhibition of Tresp cell proliferation. Data were expressed as the mean $\pm$ standard error (SEM). Data were analyzed using One Way Analysis of Variance or Kruskal–Wallis One Way Analysis of Variance on Ranks as appropriate, followed by Student–Newman–Keuls Method for *post hoc* analysis. The null hypothesis was rejected at a 5% level.

Results

Demographics and clinical characteristics

Table 1 illustrates the features of the four groups. Mean ages are the same and BMI was higher in the Ob-C and Ob-A groups, compared to Nw-C and Nw-A groups. The BMI was similar between Ob-C and Ob-A groups. As expected, both lean and obese asthmatics had significantly lower FEV1% predicted, compared to controls. Subjects in the Ob-A group had an even lower FEV1% predicted and worse asthma control and quality of life, compared to Nw-A group. All asthmatics reported as needed use of a short-acting beta agonist. Approximately 60% of all asthmatics said that they used inhaled corticosteroids (ICS) at least once daily.

Table 1. Characteristics of the Four Groups.

	Normal weight-control (n = 17)	Obese-control (n = 17)	Normal weight-asthma (n = 13)	Obese-asthma (n = 17)
Age (years)	33.8 $\pm$ 8	36.1 $\pm$ 2	33.0 $\pm$ 6	38.0 $\pm$ 11
Age of asthma diagnosis (years)	—	—	6 $\pm$ 3	8 $\pm$ 4
FEV1%predicted	95 $\pm$ 8	96 $\pm$ 9	80 $\pm$ 10 ^{1,2}	71 $\pm$ 10
BMI (kg/m ²)	22.5 $\pm$ 4	35.1 $\pm$ 4 ³	22.8 $\pm$ 3	35.9 $\pm$ 6
ACQ	—	—	0.9 $\pm$ 0.8 ⁴	1.8 $\pm$ 1
AQLQ	—	—	5.5 $\pm$ 0.7 ⁵	3.8 $\pm$ 0.8
IgE (IU/ml)	20 $\pm$ 18	15 $\pm$ 15	120 $\pm$ 70 ⁶	145 $\pm$ 80
ICS Usage	—	—	9/15 (60%)	11/17 (64%)

1 = FEV1% predicted was significantly lower in asthmatics compared to controls, 2 = FEV1% predicted was significantly higher in lean asthmatics compared to obese asthmatics, 3 = BMI was significantly higher in obese groups compared to normal weight groups. BMI was the same for obese asthmatic and obese controls. 4 = Asthma control was significantly better in lean asthmatics compared to obese asthmatics. 5 = lean asthmatics had better quality of life compared to obese asthmatics. 6 = IgE levels were higher in asthmatics compared to controls. There were no differences in ages between all 4 groups. ICS: Inhaled corticosteroid.

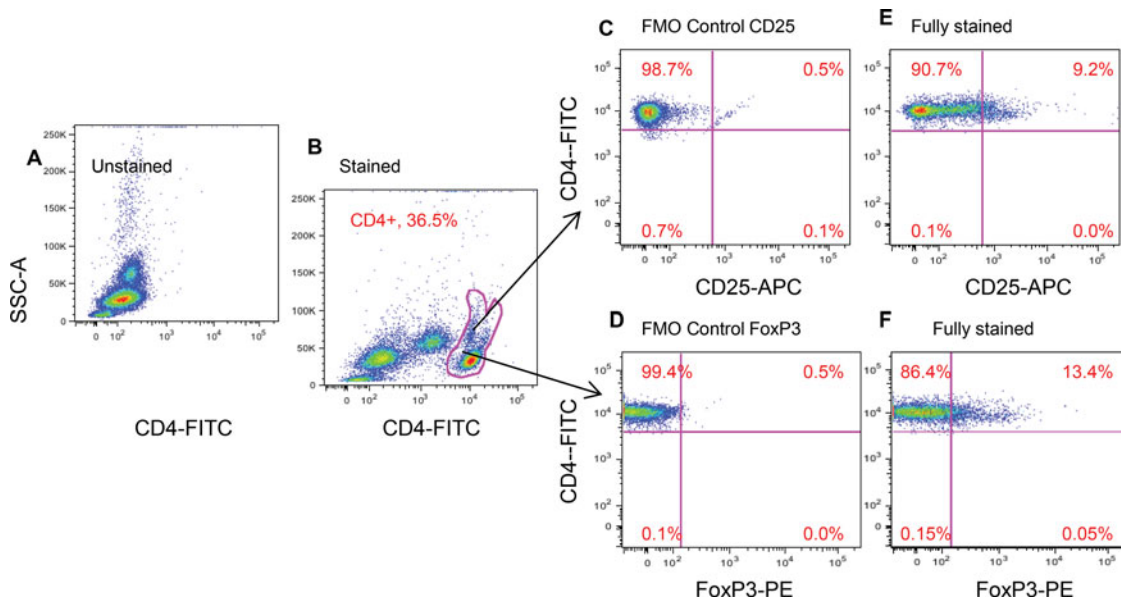


Figure 1. Representative flow cytometry dot plots illustrate the gating strategy for flow cytometric identification of CD4⁺/CD25⁺/FoxP3⁺ regulatory T (Treg) cells. Peripheral blood mononuclear cells (PBMCs) were unstained, stained with CD4-FITC, CD25-APC and FoxP3-PE antibodies or stained with all the fluorophore-conjugated antibodies minus one (CD25-APC or FoxP3-PE) in fluorescence minus one (FMO) control samples. Cells were analyzed using BD flow cytometer, and Treg cells identified and counted following the gating strategy. (A and B) Based on their forward- and side-scatter characteristics, and CD4 immunopositivity, CD4⁺ T cell population was first identified and further analyzed (C and D). Based on FMO control staining referencing to its corresponding fully stained sample, boundary line was determined, which separates CD4⁺/CD25⁺ staining (E and F) from CD4⁺/CD25⁻ cells (C) or CD4⁺/FoxP3⁺ from CD4⁺/FoxP3⁻ cells (D). The boundary line also corrects non-specific staining (E and F). Applying the boundary line to corresponding fully stained sample on CD4-FITC vs CD25-APC (E) or a CD4-FITC vs. FoxP3-PE (F) dot plot to identify CD4⁺/CD25⁺ or CD4⁺/FoxP3⁺ T cells. Finally, applying the boundary lines to fully stained sample on FoxP3-PE vs CD25-APC dot plot to identify and quantify CD4⁺/CD25⁺/FoxP3⁺ Treg cells (Figure 3A).

Increased number of peripheral blood Treg cells in obese asthmatics

To understand the potential contribution of Treg deficiency or aberrant function to the early-onset asthma obese phenotype, we first compared the numbers of peripheral blood CD4⁺/CD25⁺/Foxp3⁺ Treg cells between normal weight and obese asthmatics in reference to normal weight and obese controls. Figure 1 illustrates the gating strategy for flow cytometric identification of CD4⁺/CD25⁺ and CD4⁺/FoxP3⁺, and then CD4⁺/CD25⁺/FoxP3⁺ Treg cells within the CD4⁺ T cell population. The CD4⁺ T cells were identified based on their forward- and side-scatter characteristics, and CD4 immunopositivity (Figure 1), and quantified. Total numbers of CD4⁺ T cells were not different between any two of the four groups (Figure 2). CD4⁺/CD25⁺/FoxP3⁺ Treg cell subpopulation within the CD4⁺ T cells was subsequently identified and counted (Figure 3A) using the gating strategy illustrated in Figure 1. The number of peripheral blood CD4⁺/CD25⁺/FoxP3⁺ Treg cells as a percentage of total CD4⁺ T cells was comparable between Nw-C and Ob-C groups but was significantly reduced in Nw-A group (Figures 3A,3B,3C and 3E). By contrast, the

percentage of peripheral blood CD4⁺/CD25⁺/FoxP3⁺ Treg cells was markedly increased in Ob-A group (Figures 3A-3D and 3E).

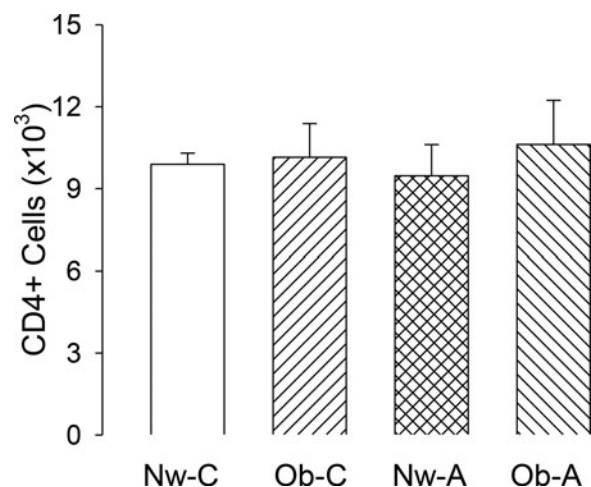


Figure 2. Bar graph shows that total numbers of peripheral blood CD4⁺ T cells were comparable among the 4 groups of subjects. PBMCs were isolated from normal weight control (Nw-C), obese control (Ob-C), normal weight asthma (Nw-A) and obese asthma (Ob-A) group of subjects, stained, analyzed and quantified using flow cytometry as described above, and expressed as $\times 10^3$. Means $\pm$ SEM of 13–17 subjects per group.

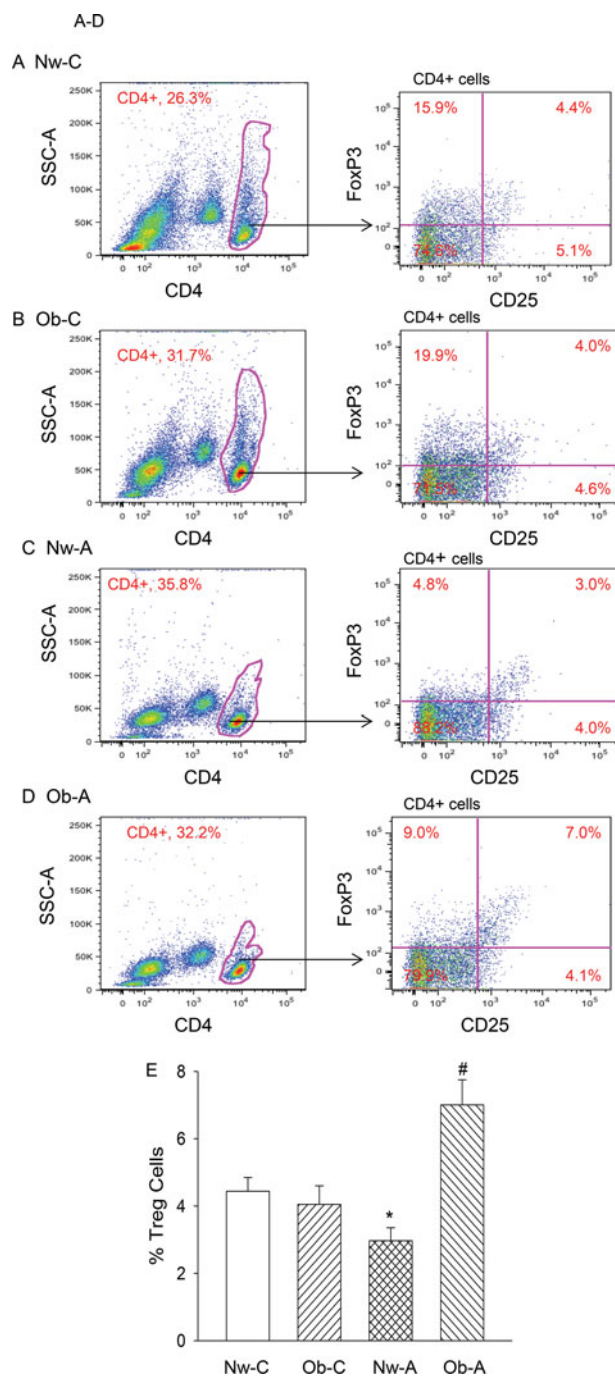


Figure 3. Obese atopic asthmatics have increased peripheral blood Treg cells. PBMCs were isolated from the 4 groups of subjects, stained and analyzed as described above. (A–D) Representative flow cytometry dot plots show an increased number of peripheral Treg cells in atopic obese asthmatics. CD4+ T cell population was first identified based on their forward- and side-scatter characteristics, and CD4 immunopositivity. Gates were then set to detect CD4+/CD25+/FoxP3+ Treg cell subpopulation using the gating strategy described in Figure 1. (E) Bar graph shows that obese asthmatics have increased peripheral blood Treg cells. CD4+/CD25+/FoxP3+ Treg cells and CD4+ T cells were quantified. Number of Treg cells was expressed as a percentage of total CD4+ T cells. Means $\pm$ SEM of 13–17 patients per group. *, $p < 0.05$, compared to Nw-C group. #, $p < 0.05$, compared to Nw-C, Ob-C or Nw-A group.

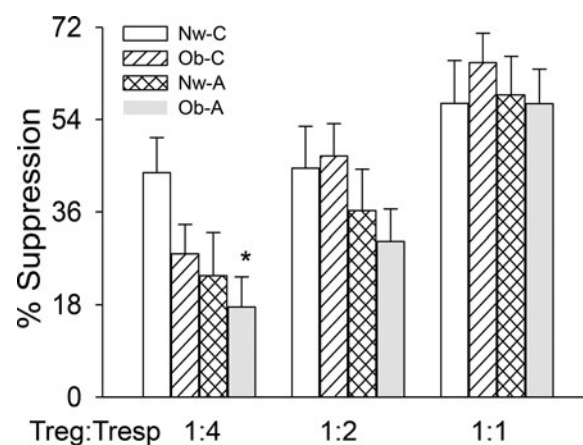


Figure 4. Obese atopic asthmatics show a mildly impaired Treg cell function. CD4+/CD25- responder T cells (Tresp) were cultured alone or co-cultured with autologous CD4+/CD25+ Treg cells at a Treg-to-Tresp ratio of 1:4, 1:2 or 1:1. Cells were stimulated with CD2, CD3 and CD28 antibodies-coated MACSibead to induce Tresp cell proliferation. Cell proliferation was measured by ^3H -thymidine incorporation. Suppression of Tresp cell proliferation by Treg cells was expressed as a percentage inhibition of Tresp cell proliferation. Means $\pm$ SEM of 13–17 patients per group. *, $p < 0.05$, compared with Nw-C group.

Comparison of Treg suppressive function between lean and obese asthmatics

A significant function for Treg cells is to suppress proliferation or downregulate induction of effector T cells. We, therefore, compared the ability of Treg cells to suppress autologous Tresp cell proliferation in response to immune stimulation among the four groups of subjects. While FoxP3 is a specific marker for Treg cells, it must be recognized that it is a nuclear protein. Therefore, it is not technically feasible to isolate viable CD4+/CD25+/Foxp3+ Treg cells for functional studies. We consequently used CD4+/CD25+ T cells as Tregs and CD4+/CD25- T cells as Tresp cells for our studies. The proportion of CD4+CD25+/FoxP3+ cells in the CD4+CD25+ subpopulation was not significantly different among Nw-C, Ob-C, and Mw-A groups, but was significantly higher in Ob-A group. Tresp cells were cultured alone or were co-cultured with autologous Treg cells at a Treg-to-Tresp ratio of 1:4, 1:2, or 1:1, and Treg suppression of Tresp cell proliferation following immune stimulation was analyzed, and compared. Tregs suppressed Tresp cell proliferation in a Treg cell number-dependent manner (Figure 4). At lower Treg-to-Tresp cell ratio (1:4) in the co-cultures, the % suppression of Tresp cell proliferation by Tregs was more modest in the Ob-C and Nw-A groups and was significantly reduced in Ob-A group (Figure 4, 1:4). The reduced suppression of Tresp cell proliferation by Treg cells became less apparent with increasing Treg-to-Tresp cell ratio in the co-cultures in all four groups (Figure 4,

1:2, 1:1). At Treg-to-Tresp cell ratio 1:1, the % suppression of Tresp cell proliferation by Treg cells was similar among the four groups (Figure 4, 1:1). These results suggest that both obesity and asthma tend to impair Treg function, and that coexistence of obesity and asthma significantly reduce Treg suppressive function. However, this impairment is mild since it can be overcome by increasing Treg cell number.

Discussion

We studied a well-characterized group of asthmatics; premenopausal women with childhood-onset, atopic obese-asthma phenotype. We found that obese asthmatics exhibit an increased number of peripheral blood CD4⁺/CD25⁺/FOX3⁺ Treg cells, while lean atopic asthmatics had fewer Treg cells. The increase in Treg cells is accompanied by a reduced Treg function. The ability of Treg cells to suppress autologous Tresp cell proliferation was significantly reduced in obese asthmatics at a Treg-to-Tresp cell ratio of 1:4. Nevertheless, this impairment appears mild, since the Treg cell suppressive function was restored to control level at the high Treg-to-Tresp ratio (1:1).

One of the important questions in human asthma is the mechanism by which obesity alters the immune responses and airway inflammation. Our classic understanding of asthma is that it is an inflammatory disease characterized by eosinophilic airway inflammation with Th2 skewing characterized by increases in Th2-related chemokines (10,22). In our study, we were interested in understanding whether differences exist in Treg cells between well-matched groups. A unique feature of our study is concentrating on a distinct phenotype of asthma-obesity. Prior studies investigating Treg cells in the obese asthmatic had included all obese asthmatics and a mixture of ages and genders. To our knowledge, ours is the first study to analyze Treg number and function in female adult atopic obese asthmatics whose asthma was diagnosed at childhood. Our data represent an essential addition to the current knowledge of pathogenic mechanisms of this obese atopic asthma phenotype. In our study, we included the appropriate controls by assessing groups of normal BMI subjects with and without asthma. Since there appears to be a dose-response effect of obesity on asthma, we studied a group of individuals that was clearly obese with BMI ≥ 35 kg/m², which is above the threshold used to define obesity (22). We reasoned that this would maximize our ability to observe differences between asthmatics and non-asthmatics. We further reduced confounding factors by studying only adult premenopausal women. Furthermore, obese asthmatics had more severe

asthma as indicated by a lower FEV1% predicted and ACQ and AQLQ scores than lean atopic asthmatics.

Our results demonstrate that normal weight, atopic women whose asthma was diagnosed in childhood had reduced numbers of peripheral blood Treg cells. In contrast, obese women also with atopy, similar age, and early-onset asthma, had increased Treg cells in their peripheral blood. Our data suggest that the reduced Treg cell number is not a contributor to the atopic obese-asthma phenotype. We speculate that the increased number of Treg cells may serve as a compensatory mechanism counteracting immune dysregulation and inflammatory response. The mild impairment in Treg function could be a contributor to the more severe asthma in the obese atopic individual with a childhood diagnosis of asthma. Alternatively, the reduced Treg function may act as a trigger, which in turn up-regulates Treg cell generation, leading to an increase in Treg number to compensate for the decreased capacity.

A prior study by Donma and colleagues (20) reported a lower number of peripheral blood Treg cells in obese asthmatic children, compared to age-matched lean children. These prior results do not necessarily conflict with our data. First, the previous study showed that peripheral blood Treg cell number was approximately 93% and 16%, respectively, higher in the obese asthmatic group than in obese control and lean asthmatic groups, which is in line with our observation. Second, in the study by Donma and colleagues (20), Treg numbers appear to be lower in all groups when compared to results reported by other investigators (15,18). Additionally, Donma et al. (20) studied children of both sexes, whereas we examined only adult females who have had asthma for decades. The immune system and the underlying pathological conditions are undoubtedly different between the children and adults.

Limitations

We studied Treg cells in peripheral blood samples of asthmatic and controls. Treg cells may differ between the peripheral blood and the site of inflammation (23). Further studies are needed to examine Treg cells in bronchial biopsies or bronchoalveolar lavage. As noted, we concentrated on one phenotype of obese-asthmatics. Therefore, our results do not extend to other phenotypes, sex or age. Leptin is a proinflammatory adipokine and is elevated in obese persons. Leptin could modulate AHR (22) and Treg function (24). We did not measure serum leptin in our study since we were interested specifically in Treg cells. Furthermore, leptin appears to play a role in the adult-onset obese asthma Th2-independent phenotype (10,22). We studied obese asthmatics with early-onset asthma

with a history of atopy, whose asthma is thought to be Th2-mediated. Further studies are required to elucidate the role of leptin in this Th2-phenotype. While none of the asthmatics enrolled in this study was taking oral corticosteroids, about 60% were reportedly on maintenance ICS. Therefore, it is possible that ICS may have modified Treg number and function. The effect of ICS on Treg cells is not fully understood. Mouse models of asthma show either no effect or an increase in Treg cells (25). Provoost et al. (15) demonstrated increased FOXP3 mRNA expression in peripheral blood steroid treated vs. untreated asthmatics. However, the asthmatics in this study received both inhaled and oral corticosteroids. In our study, the frequency of ICS usage was similar in the two asthmatic groups (about 60%). However, we found that in lean asthmatics, Treg cells were decreased while Treg cells were increased in the obese asthmatics. We, therefore, cannot attribute the changes in Treg cells solely on the effects of ICS. Further studies are required to assess the impact (if any) of ICS on Treg number and function in human asthma. Finally, we did not perform overnight sleep studies in obese individuals as this was beyond the scope of our research. While we cannot entirely exclude the presence of OSA in our obese subjects, we excluded those with a known diagnosis of OSA and excluded those with a score of ≥ 3 on the Stop-Bang sleep questionnaire, which is reported to have the highest sensitivity among sleep screening questionnaires (19).

Conclusion

The subtype of obese asthmatics with atopy, whose asthma was diagnosed in childhood has increased Treg cells in peripheral blood and a mild decrease in Treg function. This obese-asthma Treg profile differs from that of lean atopic women whose asthma was also diagnosed in childhood. This group of lean asthmatics had reduced numbers of peripheral blood Treg cells. Our results suggest that the changes in Treg cells in this phenotype may be a consequence rather than a cause of more severe asthma in this population. We propose that an increased number of Treg cells is a compensatory mechanism to dampen the robust inflammatory response in this obese atopic asthma phenotype. A better understanding of the role of Treg cells in the obesity-asthma phenotype may lead to improved therapy.

Declarations of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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