ORIGINAL ARTICLE

Regulatory T Cells in Elderly Patients With Asthma

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Abstract

Background: Airway walls in asthma present an accumulation of activated cells that determine bronchial structural changes and disease progression and severity. During the aging process, the immunoinflammatory response changes as a consequence of chronic antigenic stress.
Objective: To evaluate T-cell subsets with regulatory functions associated with asthma in elderly patients.
Methods: A group of 153 individuals (95 with controlled asthma and 58 healthy controls) aged over 65 years was studied. Blood samples were collected for flow cytometry analyses of CD3, CD4, CD8, CD56, CD56CD8, CD3CD4CD25, CD3CD4CD25CD127, CD4HLA-DR and TCR$\gamma\delta$.
Results: Asthmatic patients showed a statistically significant increase in $CD4^+$ T cells. $CD3CD4CD25^{high}$ and $CD3CD4CD25^{high}CD127^{high}$ cells were also significantly increased in asthmatic patients, while $CD3CD4CD25^{high}CD127^{low}$ cells had similar values in asthmatics and in the control group. CD4HLA-DR cells were within the normal range in both groups. A positive correlation between $CD3CD4CD25^{high}CD127^{low}$ and CD4HLA-DR was observed and $\gamma\delta$ T cells were significantly decreased in the asthmatic patients compared to the controls.
Conclusions: Since T cells with regulatory functions were within normal ranges or reduced in asthmatic patients compared to healthy controls, at least in basal conditions, it can be speculated that they probably play a limited role in chronic asthma in elderly patients. These data suggest an absence of a modulatory effect on the inflammatory response that characterizes asthma and allergy, which in turn would facilitate the persistence of disease in this population. Underlying inflammatory processes that are involved in chronic diseases associated with aging could provide an additional explanation for the attenuated differences observed between asthmatic and nonasthmatic individuals.

Key words: Asthma. Elderly. Treg cells. T cells.

Resumen

Antecedentes: En el asma, las paredes de las vías respiratorias presentan una acumulación de células activadas que determinan las alteraciones bronquiales estructurales, así como la progresión y la gravedad de la enfermedad. Durante el proceso de envejecimiento, la respuesta inmunoinflamatoria se altera a causa del estrés antigénico crónico.
Objetivo: Evaluar los subconjuntos de linfocitos T con funciones reguladoras asociados con el asma en pacientes ancianos.
Métodos: Se estudió a 153 personas (95 con asma controlada y 58 controles sanos) mayores de 65 años. Se obtuvieron muestras de sangre para análisis de citometría de flujo de CD3, CD4, CD8, CD56, CD56CD8, CD3CD4CD25, CD3CD4CD25CD127, CD4HLA-DR y TCR$\gamma\delta$.
Resultados: Los pacientes asmáticos mostraron un aumento estadísticamente significativo de los linfocitos T $CD4^+$. Los linfocitos $CD3CD4CD25^{high}$ y $CD3CD4CD25^{high}CD127^{high}$ también aumentaron significativamente en los pacientes asmáticos, mientras que los valores de linfocitos $CD3CD4CD25^{high}CD127^{low}$ fueron similares en los pacientes asmáticos y en el grupo de control. Los linfocitos CD4HLA-DR estuvieron dentro del intervalo normal en ambos grupos. Se observó una correlación positiva entre los linfocitos $CD3CD4CD25^{high}CD127^{low}$ y CD4HLADR, y los linfocitos T $\gamma\delta$ disminuyeron significativamente en los pacientes asmáticos en comparación con los controles.
Conclusiones: Dado que los linfocitos T con funciones reguladoras estuvieron dentro de los intervalos normales o disminuyeron en los pacientes asmáticos en comparación con los controles sanos, al menos en las condiciones iniciales, es probable que tengan un papel limitado en el asma crónica en pacientes ancianos. Estos datos indican la ausencia de un efecto modulador sobre la respuesta inflamatoria que caracteriza al asma y la alergia, lo que a su vez facilitaría la persistencia de la enfermedad en esta población. Los procesos inflamatorios subyacentes que intervienen en las enfermedades crónicas asociadas con el envejecimiento proporcionan una explicación adicional para las diferencias atenuadas observadas entre personas asmáticas y no asmáticas.

Palabras clave: Asma. Ancianos. Linfocitos Treg. Linfocitos T.

Introduction

Asthma is a chronic inflammatory disorder of the airways characterized by widespread but variable bronchial obstruction and hyper-responsiveness to several triggers. Asthmatic airway walls present an accumulation of activated eosinophils, lymphocytes, mast cells, macrophages, dendritic cells, and myofibroblasts, which, combined, determine bronchial structural changes and disease progression and severity [1]. Lung injury is a result of the balance between aggression and repair achieved through defence mechanisms that depend on the modulation of regulators [2]. The immunoinflammatory response changes during the aging process, assumedly as a consequence of the continuous damage caused by chronic antigenic stress throughout life.

T cells and B cells are responsible for specific responses to antigen and have a central role in the immune system. Antigen stimulation of $CD4^+$ T cells results in the activation and differentiation of these cells into several functional subsets [3]. Recent studies in T-cell biology have revealed a subset of $CD4^+$ T cells that constitutively express high levels of CD25 (the interleukin [IL] 2 receptor α chain: $CD4CD25^{high}$ T cells) and the forkhead transcription factor FoxP3. These $CD4CD25^{high}$ cells can inhibit the activation of allergen-responsive T cells but this inhibitory effect is reduced in atopic patients [4]. These cells, known as regulator T cells, or Tregs, seem to play a central role in regulating inflammation [4]. Moreover, uncontrolled pediatric asthmatics have lower $CD4^{low}CD25^{high}$ T cells in bronchoalveolar lavage fluid than children treated with corticosteroids or healthy children [5]. The best cell surface activation marker is CD25, which is expressed not only in Treg cells but also in activated cells without regulatory functions. CD127, the α-chain of the IL-7 receptor, is expressed on mature T cells and plays an important role in their proliferation. Since CD127 is absent on regulatory T cells and its expression is inversely correlated with FoxP3 expression, regulatory $CD4^+$ T cells expressing CD25 and/or $CD25^{high}$ can be distinguished by a reduced expression of CD127. There is increasing evidence of an age-dependent development of Tregs. The number of $CD4CD25^{high}$ T cells in healthy individuals increases with age, particularly after 60 years. Accumulation of memory-like CD45R0 Tregs may account for the trend towards increased $CD4CCD25^{high}$ Tregs observed in some studies of the elderly [6] and peripheral Treg production is likely to increase with the involution of the thymus [7,8]. According to in vitro studies, Treg cells have similar patterns of response in younger and older age groups [6]. Chronic quiescent inflammation, which is a recognized characteristic of elderly people, may promote the occurrence of Treg cells, which may, in turn, facilitate a decline in adaptive response. This decline is characteristic of immunosenescence and is related to the gradual changes that occur in the immune system with age. These changes may be responsible for the frequent occurrence of immunoinflammatory respiratory diseases in the aging population [6,9,10].

Natural killer (NK) cells play an important role in the destruction of antigens and in the regulation of immune responses in a broad range of diseases including cancer. NK T (NKT) cells (CD56CD3) constitute a small subgroup of NK cells that express surface receptors of both T cells and NK cells [11,12]. Most (>75%) NKT cells are $CD8^+$ and produce type 1 T helper (T_H1) cytokines, with a regulatory effect on allergic inflammation. The CD8CD56 NKT cell phenotype displays both antigen-specific cytolytic T cell activity, as well as NK-like cytolytic activities, which are independent of human leukocyte antigen class I and CD1 molecules. This major subset of NKT cells has potent cytolytic and immunoregulation functions. The elderly have elevated NKT cells and in particular an elevated NKTCD8 subpopulation [13].

T cells committed to $\gamma\delta$ T cell receptor (TCR$\gamma\delta$) expression are present in the airways, where they offer important protection against infecting microorganisms. These $\gamma\delta$ cells account for 1% to 10% of circulating mature lymphocytes and have been identified in the airways of patients with rhinitis and asthma, suggesting that they might be involved in allergic airway inflammation [14]. It has also been demonstrated that TCR$\gamma\delta$ T cells can inhibit late airway allergic responses through the release of interferon (IFN) γ, playing an important role as regulators of airway inflammation in immunoglobulin E– mediated diseases [15]. Furthermore, aging is characterized by a variety of alterations in $\gamma\delta$ T-cell function and the absolute number of these circulating cells can be greatly reduced in the elderly [16,17].

The aim of this study was to compare, by flow cytometry analysis, blood T-cell subsets with regulatory tasks, such as $CD4CD25^{high}CD127^{low}$ T cells, NKT cells, and $\gamma\delta$ T cells in a group of elderly asthmatics and age-matched healthy controls.

Methods

Study Population

A group of 95 individuals aged over 65 years with controlled asthma attending an allergy clinic and an aged-matched control group of 58 healthy volunteers were included in the study (Table 1). All the patients had a history of

Table 1. Sex and Age Distribution in Elderly Asthmatics and Age-Matched Controls

Population	No.	Men/Women, No.	Age, Mean (SD), y	Age Range, y
Control group	58	19/39	79 (7)	65-94
Asthmatic patients	95	32/63	72 (5)	65-87

intermittent chest tightness, wheezing, or shortness of breath for at least 30 years prior to study enrolment consistent with the diagnosis of asthma according to the Global Initiative for Asthma (GINA) guidelines [18]. Patients with mild/moderate asthma were taking beclomethasone dipropionate (dose range, 250-500 μg) and those with severe asthma were taking fluticasone propionate (250 μg) in association with long-acting ß$_2$-agonists on a daily basis and short-acting ß$_2$-agonists as needed. All other antiasthmatic drugs were withdrawn at least 4 weeks prior to the study.

All patients were examined by a physician and underwent spirometry using the same equipment (Vitalograph Compact, Ennis, Ireland) at least 6 hours after the last dose of any bronchodilator. Predicted values were measured according to Knudson et al [19]. Spirometric performances were assessed by means of a computerized program following the American Thoracic Society (ATS) 94 criteria [20-22]. Approval for analysis was determined using the ATS 94 criteria and accuracy was achieved if 3 curves within the same evaluation were acceptable and reproducible.

All the patients and controls were nonsmokers, able to perform their own daily tasks, and physically active on a regular basis. None had had any respiratory infections in the month prior to inclusion in the study. No other clinically relevant diseases were reported. The following were considered exclusion criteria: cancer, autoimmune disease, infection, diabetes, heart failure, renal failure, chronic hepatic disease, and recent exposure to environmental risk factors for pulmonary diseases. Informed consent was obtained from all participants before enrolment.

Flow Cytometry

The study of peripheral blood (PB) lymphocytes was performed using flow cytometry. Four milliliters of peripheral blood was drawn from the vein of the forearm into a tube with anticoagulant; 100 μL of PB was then incubated with monoclonal antibodies for 10 minutes at room temperature in the dark.

To determine the main populations, a single tube with Lymphogram (Cytognos, Salamanca, Spain) containing anti-CD4 monoclonal antibodies marked with phycoerythrin cyanin 5 (Pecy5), CD8/CD19 stained with fluorescein-isothiocyanate, and CD3/CD56 with phycoerythrin was used. The percentages of CD4$^+$ or CD8$^+$ cells are referred to as the CD3$^+$ subset after the lymphocyte gate and the CD56$^+$ cells are referred to as the CD3$^+$ negative subset. The population analyzed (defined as CD56CD8) comprised CD56$^+$ NK cells that co-expressed CD8. HLA-DR, TCR γδ (both from Immunotech, Marseille, France), CD8 (Dako, Denmark), and CD127 (BD Pharmingen, San Jose, California, USA) were stained with phycoerythrin. CD3 and CD4 with PeCy5 (Dako, Denmark), CD3 APC (Allophycocyanin), CD4 Percp (Peridinin chlorophyl protein) (BD Biosciences, San Jose, California, USA), and CD25 fluorescein-isothiocyanate (Sanquin, Amsterdam, The Netherlands) were used according to the manufacturer's instructions. The expression of CD25 and CD127 was analyzed using a lymphocyte gate.

Flow cytometry data were collected on a FACSCalibur flow cytometer (BD Biosciences) using CellQuest acquisition software and analyzed by Paint-a-gate software.

Statistical Analysis

Statistical analysis was performed using SPSS 12.0 software. The Kolmogorov–Smirnov test was used to check for normal distribution. As all the variables were normally distributed the parametric t test was used to compare pairs of independent samples. *P* values of lower than .05 were considered significant. Statistical comparisons were made between elderly asthmatics and healthy age-matched individuals. Differences between severe and mild/moderate asthmatic patients were also analyzed.

Results

All the patients included in the study were clinically stable and 19 had a predicted forced expiratory volume in the first second (FEV_1) of less than 60%; 76 patients had a FEV_1 of above 60%. The 19 patients were considered to have severe asthma and the remaining 76, mild or moderate asthma. The average lung function values of these 2 groups are shown in Table 2.

The mean percentages of the main blood cells subsets, CD3$^+$, CD4$^+$, and CD8$^+$ T cells, were within normal ranges, although CD4$^+$ cells were significantly increased in asthmatic patients. The increase was more evident in the subgroup of patients with mild/moderate asthma (Figure 1 and Table 3).

The percentage of NK cells expressing CD56 in asthmatic patients was slightly decreased but accounted for more than 15% of PB mononuclear cells analyzed. The percentage of

Table 2. Predicted Spirometric Values[a] in Elderly Asthmatic Patients

	No. of Patients	%FEV_1	FEV_1% after BD	%FVC	Tiffeneau Index
Severe asthma	19	51.8 (10.0)	10.2 (3.7)	68.1 (10.2)	70.1 (9.7)
Mild/moderate asthma	76	87.6 (17.8)	12.4 (6.2)	94.5 (15.5)	83.5 (13.0)

Abbreviations: BD, bronchodilation; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity;
[a]Predicted values were measured according to Knudson [16] and are expressed as mean (SD).

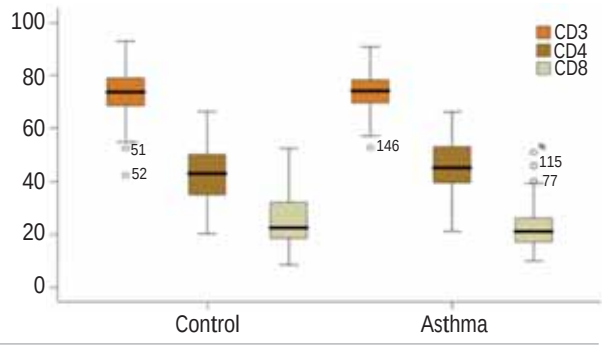

Figure 1. The mean (SD) percentage of CD3$^+$ T cells in asthmatic patients (73.4% [7.6%]) was similar to that observed in the control group (73.6% [9.9%]). CD4$^+$ T cells were significantly increased in asthmatic patients (45.4% [9.6%]) compared to the control group (41.8% [10.4%]) (P<.05). CD8$^+$ T cells were slightly but not significantly lower in asthmatic patients (22.9% [8.0%]) compared to the control group (25.0% [9.5%]).

NKT cells (CD56CD8) was also slightly decreased in asthmatic patients but no significant differences were observed between groups (Figure 2 and Table 3).

Previous studies have analyzed the role of CD4CD25^{high} T cells in immune regulation [2,4]. In our study, the percentage of CD3CD4$^+$ cells expressing the IL-2 receptor (CD3CD4CD25^{high} T cells) was significantly increased in patients with both severe and mild/moderate asthma (Figure 3 and Table 3). Furthermore when the expression of CD127 in these subsets was tested, the percentage of CD3CD4CD25^{high}CD127 and CD3CD4CD25^{high}CD127^{high} T cells was also significantly increased in all asthmatic patients studied. On the contrary, the percentage of CD3CD4CD25^{high}CD127^{low} T cells, which were recently connected with a more accurate identification of regulatory activity [21], was similar in asthmatic patients and in the control group. Within the group of asthmatic patients, disease severity did not alter the above results (Figure 4, Figure 5, and Table 3).

Table 3. Cell Population Distribution According to Asthma Severity

	Healthy Controls, %	Patients With Severe Asthma, %	Patients With Mild/Moderate Asthma, %
CD3	73.6 (9.9)[b]	74.2 (9.2)[b]	73.2 (7.5)[b]
CD4	41.8 (10.4)[b]	44.7 (10.0)[b]	45.6 (9.2)[c]
CD8	25.0 (9.5)[b]	24.8 (8.8)[b]	22.4 (7.7)[b]
CD56	16.2 (8.7)[b]	15.9 (8.3)[b]	14.8 (6.5)[b]
CD56CD8	5.9 (4.5)[b]	5.5 (2.4)[b]	5.4 (3.3)[b]
CD3CD4CD25^{high}	4.4 (1.0)[c]	5.7 (2.1)[c]	5.4 (1.7)[c]
CD3CD4CD25^{high}CD127	2.8 (0.9)	4.7 (2.5)[c]	4.1 (1.6)[c]
CD3CD4CD25^{high}CD127^{high}	1.7 (0.7)	2.9 (2.1)[c]	2.8 (1.2)[c]
CD3CD4CD25^{high}CD127^{low}	1.2 (0.4)	1.6 (1.0)[b]	1.3 (0.5)[b]
CD4HLA-DR	3.2 (1.7)	3.9 (4.0)[b]	3.9 (3.6)[b]
γδ	4.1 (3.3)	2.7 (2.1)[b]	3.0 (2.8/)[b]

[a]CD4 T cells were significantly increased in mild/moderate asthmatic patients. No significant differences in CD56 and CD56CD8 cells were observed between patients with mild/moderate or severe asthma and healthy controls. CD3CD4CD25 T cells were significantly increased in patients with severe and mild/moderate asthma. CD3CD4CD25CD127 T cells and CD3CD4CD25^{high}CD127^{high} T cells were significantly increased in patients with severe and mild/moderate asthma. No significant differences in CD3CD4CD25^{high}CD127^{low} or γδ T cells were observed between patients with mild/moderate or severe asthma and healthy controls.
[b]Statistically significant difference (P<.05)
[c]No statistically significant difference.

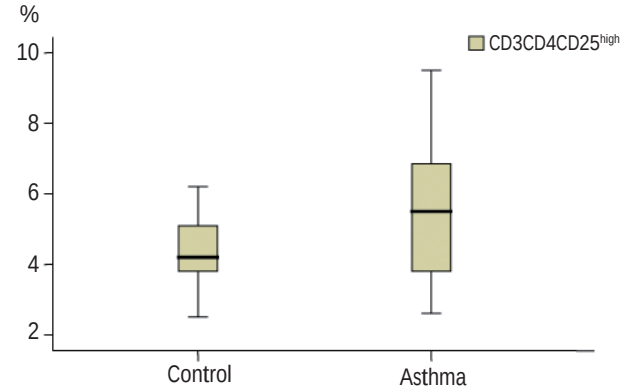

Figure 3. The mean (SD) percentage of CD3CD4CD25^{high} T cells in asthmatic patients was significantly increased (5.5% [1.9%]) when compared to the control group (4.4% [1.0%]) (P<.05).

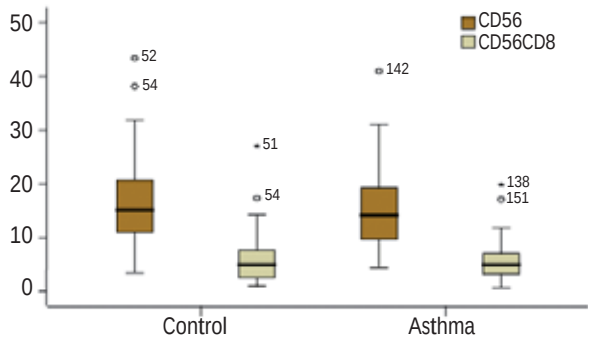

Figure 2. The mean (SD) percentage of CD56$^+$ T cells in asthmatic patients was slightly decreased (15.0% [6.9%]) when compared to the control group (16.2% [8.7%]). CD56CD8$^+$ T cells were also decreased in patients with asthma (5.4% [3.2%]) when compared to the control group (5.9% [4.5%]) (not significant).

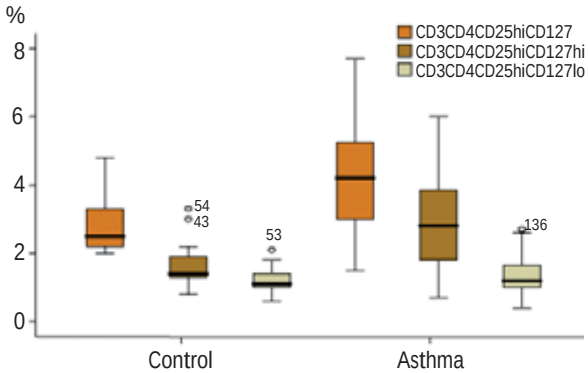

Figure 4. The mean (SD) percentage of CD3CD4CD25^{high}CD127 and CD3CD4CD25^{high}CD127^{high} T cells was significantly increased in asthmatic patients (4.2% [1.7%] and 2.8% [0.9%], respectively) when compared to the control group (1.7% [0.7%]) (P<.05). CD3CD4CD25^{high}CD127^{low} T cells were not significantly different between asthmatic patients (1.4% [0.6%]) and the control group (1.2% [0.4%]).

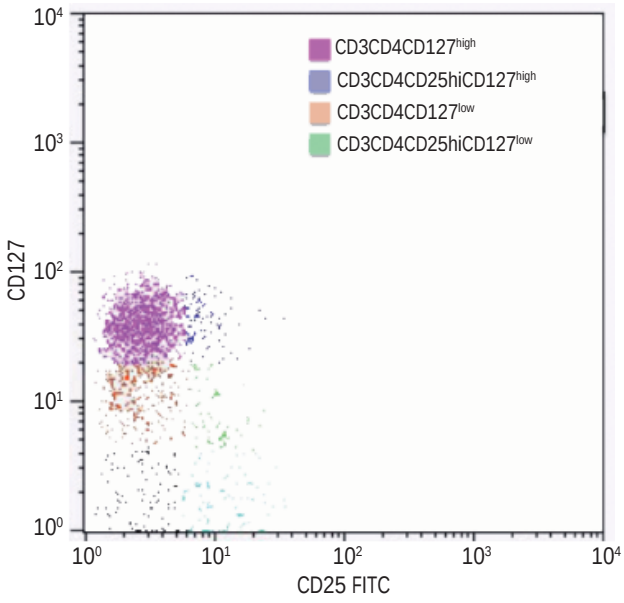

Figure 5. Flow cytometry analysis of CD3CD4CD25^{high}CD127^{low} and CD3CD4CD25^{high}CD127^{high} T cells.

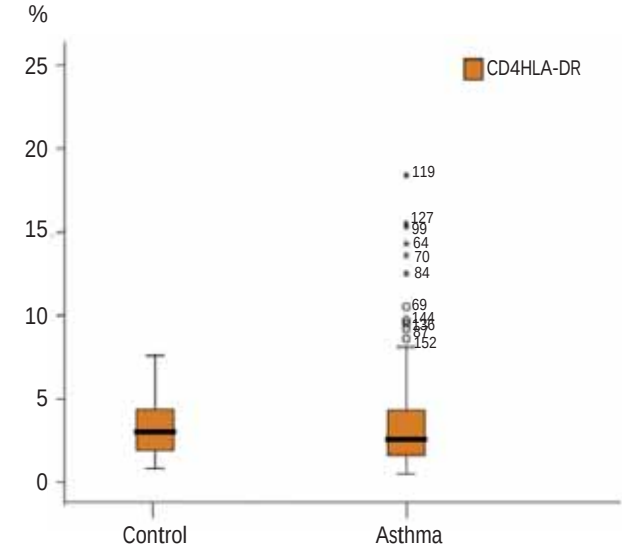

Figure 6. The mean (SD) percentage of CD4HLA-DR T cells in asthmatic patients were slightly increased (3.9% [3.7]%) when compared to the control group (3.2% [1.7%]) (not significant).

CD4HLA-DR T cells, which are believed to be upregulated during the cell activation process and to facilitate cell-to-cell communication were only slightly increased in the patient group (Figure 6 and Table 3).

The small subset of γδ T cells that do not express the classical αβ receptors were significantly decreased in asthmatics (Figure 7 and Table 3).

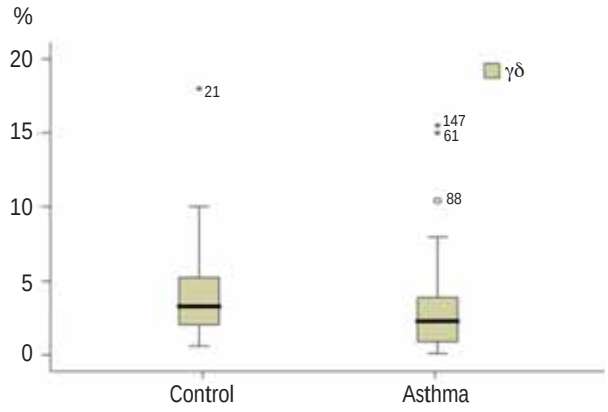

Figure 7. The mean (SD) percentage of γδ T cells in asthmatic patients was significantly decreased (2.9% [2.7%]) when compared to the control group (4.1% [3.3]) (*P*<.05).

Discussion

Asthma is a chronic inflammatory disease of the airways that affects all age groups. While some patients experience complete recovery and resolution of symptoms, others have partial remission or develop progressive and irreversible obstruction of the airways, which, in extreme cases, can cause death. Asthma in the elderly is a disease with increasing prevalence in developed countries, where people over 65 years account for over 20% of the population. The disabilities associated with this condition include not only respiratory distress but also cardiovascular events. Uncontrolled systemic inflammation is claimed to be a major factor in the high morbidity and mortality observed [22]. The identification of different regulatory cells that could dampen the immune-inflammatory process has opened several fields of investigation in these areas.

T cells are the main modulators of inflammatory response in asthma and play a key role in the eosinophilic inflammation that characterizes the disease. Activated T cells can migrate into the airways and memory T cells then react to specific inhaled environmental antigens

The asthmatic patients analyzed in our study presented a significant increase in CD4$^+$ T cells compared to the healthy control group (*P*<.05). This may be associated with disease activity despite the long duration of the disease in the patients studied [1]. The slight decrease in CD8$^+$ T cells in asthmatic patients reflects the modest participation of these cells in asthma and their minor modulatory function [23].

Although it is widely accepted that T_H2 cells are central to the pathogenesis of asthma, some studies have proposed that other T-cell subpopulations such as NKT cells may also play an important role in asthma [11,13]. NKT cells are usually well preserved throughout life, suggesting that in elderly individuals, these cells are kept at a high level of efficiency, contributing to successful aging. NKT cells co-express a TCR and a variety of characteristic molecular markers, with major histocompatibility complex restriction. The NKT cell cytokine environment can determine the development of immune

regulatory activity (IL4) or dominant proinflammatory IFN-γ activity [24]. $CD56CD8^+$ cells tend to increase with aging and in several chronic inflammatory conditions such as neoplastic diseases or autoimmunity [25-27].

In the present study, $CD56CD8^+$ cells were slightly reduced in asthmatic patients, with values of 5.4%, but they did not differ significantly from the values observed in healthy age-matched individuals. These data suggest that $CD56CD8^+$ cells do not induce an eventual regulatory function in the inflammatory process and do not assume a determinative role in chronic asthma affecting aged patients.

IL2, the greatest growth factor of T cells and an important marker of T-cell activation, regulate the expression of CD25 (IL-2Rα). Recent advances in T-cell biology have led to the identification of a subset of $CD4^+$ T cells that constitutively express CD25 and FoxP3. These $CD4CD25^{high}$ cells, denoted Tregs, are involved in regulating and suppressing inflammation [28]. Adoptive transfer of antigen-specific Tregs into allergen-sensitized mice has been seen to prevent allergic airway inflammation and airway hyperresponsiveness [29]; these cells are therefore recognized as having the capacity to exert a suppressive effect on other cells of the immune system, maintaining tolerance in the periphery [30]. Natural regulatory cells have a minor presence in adults. Their suppressive response depends on the intensity of the stimulus generated and strong stimuli can overcome Treg cell surveillance. Natural Treg cells share many characteristics with activated cells, and activated $CD4^+$ cells can positively regulate FoxP3 and acquire characteristics of regulatory cells [30]. The thymic involution associated with age, however, restricts the production and delivery of these cells.

In humans, $CD4CD25^{high}$ cells have a relatively long survival in spite of having short telomeres and limited proliferation. Indeed, they seem to be more resistant to apoptosis. The increased survival of regulatory cells leads to their accumulation in the elderly. In vitro studies have shown that these cells in cultures have equivalent suppressive activity in the young and in the elderly [6,9,31]. According to some authors, the proinflammatory environment can provide an important signal for the stimulation of regulatory cells, which also contributes to the immune suppression that characterizes the aging process [6,31].

In this study, a statistically significant increase in $CD3CD4CD25^{high}$ cells was observed in the group of elderly patients with asthma. CD25 upregulation can also occur in activated cells without regulatory functions [32]. The transcription factor Foxp3 has been reported to play a key role in $CD4CD25^{high}$ regulatory T cell function and represents a specific marker for these cells [33]. However, Foxp3 is a nuclear protein and is of limited value in the isolation of Tregs. In this study, we characterized regulatory $CD4^+$ T cells expressing $CD25^{high}$ by their low expression of CD127. $CD127^{low}$ cells are suppressive with a low proliferative response to stimulation of TCR and rapid enhancement of FoxP3. Like other γ-chain cytokines, IL-7 confers optimal suppressive activity and maintains high FoxP3 expression of Tregs in vitro [33]. There are $CD4CD25^{high}CD127$ T cells that express FoxP3. Nevertheless, after stimulation, the expression of CD127 decreases whereas that of FoxP3 increases, leading to the acquisition of suppressive ability [34,35]. The group of elderly asthmatic patients studied had increased values of $CD3CD4CD25^{high}CD127$ and $CD3CD4CD25^{high}CD127^{high}$ cells, and the differences were statistically significant when compared to the control population. The numbers of $CD3CD4CD25^{high}CD127^{low}$ cells, considered to be regulatory cells, did not differ significantly between asthmatic patients and healthy individuals. As CD127 may be specifically downregulated by FoxP3, its role in Treg homeostasis may be considered essential. Accordingly, it has been speculated that T cells with regulatory functions are not different in controlled asthmatics and healthy individuals, even when younger age groups are analyzed, at least in basal conditions, without exposure to common antigen [36]. This work follows the theory that even though activated $CD4^+$ T cells may participate in chronic asthma when $CD3CD4CD25^{high}$ cells acquire characteristics of suppressive activity, they do not necessarily play an important role in this clinical condition, at least not in elderly patients.

In our study, CD4HLA-DR cells were slightly though not significantly increased in asthmatic patients compared to controls. Regulatory T cells that express HLA-DR have a more immediate and effective function in this task through direct contact between cells [37]. A positive correlation (Pearson correlation coefficient =0.46, $P<.05$) between $CD3CD4CD25^{high}CD127^{low}$ cells and HLA-DRCD4 cells was observed in the group of patients. This study shows that patients with increased numbers of $CD4^+$ T cells expressing $CD25^{high}CD127^{low}$ cells also have increased numbers of $CD4^+$ T cells expressing HLA-DR. These data allow us to speculate that, as previously suggested, the suppressive activity of $CD4^+$ T cells might be enhanced by the expression of HLA class II molecules [38].

Although γδ T cells have been studied in detail over the last decade, their functionality and repertoire of antigenic activation have not yet been completely clarified. γδ T-cell function contributes to cellular repair processes, which mainly depend on the surrounding environment. In asthma, γδ T cells have predominantly anti-inflammatory properties [14,39]. It has been demonstrated that stimulation of bronchial tissue with anti-TCR γδ does not trigger the production of IL-5 and the ability of γδ T cells to protect against bronchial hyperreactivity has also been documented [40]. The infectious processes trigger rapid and intense γδ T-cell activity, with inhibitory effects on the synthesis of IgE. These cells tend to decrease with age and this change is closely linked to increased susceptibility to the occurrence of infections, autoimmune diseases, cancer, and chronic inflammatory processes characteristic of the elderly [41].

In this study, γδ T cells were significantly reduced in patients with chronic asthma compared to the control group, which maintained a mean (SD) value of 4.0% (3.3%) ($P<.05$). The decrease observed reflects the absence of a modulatory effect on inflammatory response, which in turn, would facilitate the persistence of disease. Immunosenescence, which refers to natural immuno-inflammatory changes that occur during the aging process, is assumed to be a consequence of the continuous damage caused by chronic antigenic stress throughout life. In this context it is accepted that the aging process underlies a

chronic inflammatory status commonly termed inflammaging. The reduced ability to downregulate the inflammation that characterizes asthma and allergy is considered to contribute to the persistence of disease and suggests that the intervention of regulatory cells in chronic asthma in the elderly is moderate.

In conclusion, asthma is a chronic inflammatory disease of the airways characterized by eosinophil accumulation directed by T_H2 cells that are recruited into the airways. However, other T-cell subsets such as NKT cells, Tregs, and γδ cells, which are widely accepted to exert a regulatory function, have been implicated. The exact function of these cells in asthma pathogenesis remains unclear. This study performed in elderly patients with asthma suggests that despite the persistence of $CD4^+$ and $CD3CD4CD25^{high}$ cells in PB, the regulatory T-cell subsets investigated do not seem to have a significant presence in chronic asthma in elderly individuals. It should be emphasized that despite the large number of patients enrolled in this study, disease severity did not introduce significant differences in cell profiles. Inflammaging may provide a continuous stimulus for regulatory cell development and for different T-cell networks in different age groups. Functional cell studies in the elderly are scarce and should be encouraged to increase knowledge in this area and possibly lead to new therapeutic strategies.

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