ORIGINAL ARTICLE

Apoptosis and Asthma in the Elderly

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Abstract

Background: Asthma is a chronic inflammatory disorder of the airways. The persistence of airway inflammation depends on a decrease in apoptosis of T lymphocytes and eosinophils and survival of these activated cells. T lymphocytes expressing γδ receptors can be identified in human lungs and play an important role in immune defence against pathogens and in the regulation of chronic inflammation. Aging is associated with evidence of some immune dysregulation.
Objective: The aim of this study was to analyze the apoptosis receptors of T lymphocytes in long-lasting asthma, to establish their correlation with activation markers such as $CD25^+$ and human leukocyte antigen (HLA)-DR^+, and to analyze the γδ T cell expression in this disease.
Methods: A group of 64 individuals (group A) who had had asthma for more than 30 years (mean age [±SD] 72 ± 5 years) and 61 healthy individuals acting as controls – group B with 41 individuals (mean age 79 ± 7 years) and group C with 20 individuals (mean age 38 ± 12 years) were included in the study. All subjects underwent clinical evaluation and spirometric testing. Peripheral blood cells were stained with monoclonal antibodies anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-TCR γδ, anti-HLA-DR and anti-CD95. Statistical comparisons were performed between the asthmatics and the elderly control group and between the elderly control group and the adult control group.
Results: The average percentage of predicted forced expiratory volume in the first second was 73.6 ± 25.3. The mean values of T cell receptors for asthma group A vs elderly control group B vs adult control group C respectively, were the following: CD3, 74.9 ± 7 vs 74.8 ± 8.8 (P = ns) vs 76.7 ± 4.2 (P = ns); CD4, 48.8 ± 8.7 vs 43.5 ± 10.2 (P = ns) vs 44.8 ± 3.8 (P = ns); CD8, 23.3 ± 7.9 vs 25.7 ± 10.2 (P = ns) vs 25.6 ± 4.5 (P = ns); CD25, 14.3 ± 5.9 vs 22.4 ± 7.8 (P = .0001) vs 5.5 ± 2.4 (P = .0001); TCR γδ, 2.8 ± 2.1 vs 4.1 ± 3.3 (P<.05) vs 4.6 ± 2.1 (P= ns); HLA-DR, 18.4 ± 9.2 vs 17.8 ± 5.9 (P= ns) vs 15.4 ± 5.1 (P= ns) and CD95, 49.3 ± 13.7 vs 52.6 ± 12.1 (P = ns) vs 13.8 ± 10.8 (P = .0001).
Conclusions: The immunological and inflammatory changes related to ageing may cause an increase in CD95 and CD25 T cell expression. In asthma, blood cells may express increased activation and apoptosis markers but in elderly patients taking steroids, these receptors remain within normal ranges. The number of γδ T cells may be lower in long-lasting asthma, and have a limited modulatory effect on allergic inflammatory reactions. The evaluation of patients with long-lasting asthma should take into account the immunological and inflammatory changes present in the elderly in order to avoid results being misinterpreted.

Key words: Asthma. Elderly. Apoptosis. TCRγδ. CD25.

Resumen

Antecedentes. El asma es una enfermedad inflamatoria crónica de las vías respiratorias. La persistencia de la inflamación de las vías respiratorias depende de la reducción en la apoptosis de linfocitos T y eosinófilos y de la supervivencia de estas células activadas. Los linfocitos T que expresan receptores γδ pueden detectarse en los pulmones humanos y juegan un importante papel en la defensa inmunológica contra patógenos y en la regulación de la inflamación crónica. El envejecimiento está asociado con manifestaciones de desregulación inmunológica.
Objetivo: El propósito del estudio fue analizar los receptores de apoptosis de los linfocitos T en el asma de larga duración para establecer su correlación con los marcadores de activación como los CD25+ y HLADR+, así como analizar la expresión de los linfocitos T γδ en esta enfermedad.
Métodos: Participaron en el estudio 64 sujetos (grupo A) que habían padecido asma durante más de 30 años (edad media de 72 ± 5 años) y un grupo control de 61 sujetos sanos - grupo B con 41sujetos (edad media de 79 ± 7 años) y grupo C con 20 sujetos (edad media de 38 ± 12 años). Se realizó la evaluación clínica de todos los sujetos, así como pruebas espirométricas. Las células de sangre periférica se marcaron con anticuerpos monoclonales anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-TCR γδ , anti-HLA-DR y anti-CD95. Se llevaron a cabo comparaciones estadísticas entre el grupo de asmáticos y el grupo control de más edad, y entre este último y el grupo control adulto.

Resultados: El porcentaje medio del volumen espiratorio máximo previsto en el primer segundo fue 73,6 ± 25,3. Los valores medios de receptores de linfocitos T (asma (A) frente a grupo control de personas de más edad (B) frente a grupo control adulto (C) fueron los siguientes: CD3, 74,9 ± 7 vs 74,8 ± 8,8 (P= ns) vs 76,7 ± 4,2 (P= ns); CD4, 48,8 ± 8,7 vs 43,5 ± 10,2 (*P* = ns) vs 44,8 ± 3,8 (*P* = ns); CD8, 23,3 ± 7,9 vs 25,7 ± 10,2 (*P* = ns) vs 25,6 ± 4,5 (*P* = ns); CD25, 14,3 ± 5,9 vs 22,4±7,8 (*P* = 0,0001) vs 5,5 ± 2,4 (*P* = 0,0001); TCR γδ: 2,8 ± 2,1 vs 4,1 ± 3,3 (*P*<0,05) vs 4,6 ± 2,1 (*P* = ns); HLA-DR, 18,4 ± 9,2 vs 17,8 ± 5,9 (*P* = ns) vs 15,4 ± 5,1 (*P* = ns) y CD95, 49,3 ± 13,7 vs 52,6 ± 12,1(*P* = ns) vs 13,8 ± 10,8 (*P* = 0,0001).
Conclusiones: Los cambios inflamatorios e inmunológicos relacionados con el envejecimiento pueden causar un aumento en la expresión de linfocitos T CD25 y CD95. En el asma, las células sanguíneas pueden expresar un aumento de la activación y de los marcadores de apoptosis, pero en los pacientes de edad avanzada que toman esteroides, estos receptores se mantienen dentro de los límites de la normalidad. El recuento de linfocitos T γδ puede ser inferior en el asma de larga duración y puede tener un limitado efecto modulador en las reacciones inflamatorias alérgicas. Para evitar la interpretación errónea de los resultados al evaluar a pacientes con asma de larga duración, se deben tener en cuenta los cambios inflamatorios e inmunológicos presentes en las personas de edad avanzada.

Palabras clave: Asma. Personas de edad avanzada. Apoptosis. TCRγδ. CD25.

Introduction

Asthma is a chronic inflammatory disorder of the airways, characterized by widespread but variable bronchial obstruction and by hyper-responsiveness to several triggers in which T lymphocytes play a central role [1,2]. Asthmatic patients tend to develop progressive decline in pulmonary function that is correlated with age, sex, disease duration and severity [3]. In the airways of asthmatics helper T cells (T_H), mainly Type 2 subclasses are activated and release a wide range of cytokines, regulating the recruitment of inflammatory cells, such as eosinophils [1]. Limitation to airways flow depends partly on the effect of the localised inflammatory process.

Apoptosis plays a central role in the development, homeostasis, and function of the immune system. In normal airways, vascular smooth muscle cells and bronchial epithelium express Fas, a member of the tumour necrosis factor receptor super family (also called Apo-1 or CD95) that undergoes apoptosis upon Fas cross-linking with FasL, which is a general mechanism for bronchial homeostasis [4]. In asthma, stimuli that promote repair mechanisms may act as a switch that induces cellular proliferation and inhibits apoptosis. A prolonged survival of inflammatory cells may contribute to the respiratory symptoms. The severity of asthma is also inversely correlated with the apoptosis of the eosinophils in the airways [5].

In asthma, peripheral T lymphocytes are also characterized by a higher expression of activation markers, such as the interleukin-2 receptor α chain (CD25), and the human leukocyte antigen (HLA)-DR that is involved in antigen presentation [1,6].

Although most T lymphocytes express T-cell receptors (TCRs) composed of α and ß chains, a high percentage of T lymphocytes expressing TCRs with γ and δ chains (TCR γδ) are localised in the skin, gut, and lung [7]. These γδ T lymphocytes, which represent 1% to 10% of circulating mature T lymphocytes, play an important role in immune defence against pathogens and in the regulation of chronic inflammation [8]. Higher numbers of TCRγδ lymphocytes have been observed in the nasal mucosa of humans with allergic rhinitis and in the bronchi alveolar lavage fluid of patients with asthma. These cells can produce T_H2 cytokines under allergenic stimulation, which suggests their participation in allergic inflammation [7]. On the other hand, TCRγδ lymphocytes under microbial stimulation can be activated and produce large amounts of interferon (IFN)-γ and down regulate the inflammatory response to allergens.

Studies that evaluate long-lasting asthma can provide important information about changes occurring during disease evolution. In such studies the inclusion of elderly patients is inevitable so the changes in the immune system with aging should always be investigated.

In the elderly, the process of apoptosis is important because it allows the rapid clearance of intact senescent cells, thus providing a limited tissue injury [9]. During the aging process, the damage-induced apoptotic pathways can be differently modulated and deficient apoptotic mechanisms can contribute to disease [10]. In addition, the absolute number of circulating TCRγδ lymphocytes can be reduced in old people and centenarians as a consequence of the age-related decrease of total lymphocyte count [11-13].

The aim of the present study was to analyze the apoptosis receptor expression on T lymphocytes ($CD3^+$) in long-lasting asthma in elderly patients, and to establish a correlation with activation marker expression on T lymphocytes ($CD25^+$ and $HLADR^+$). We also aimed to analyse TCRγδ lymphocytes in view of their ability to modulate the inflammatory process in this chronic disease. Comparisons between asthmatics (group A) and healthy individuals of the same age (group B) and between healthy elderly individuals (group B) and young healthy individuals (group C) were also carried out.

Methods

Subjects

A group of 64 individuals with mild persistent asthma (group A), older than 65 years of age (mean age 72 ± 5 years), and a control group with 61 individuals were included in the

study. The healthy cohort (control group) included two sub groups: the first with 41 elderly individuals (group B) (mean age 79 ± 7 years) and the second with 20 young adults (mean age 37.8 ± 12.2 years) (group C). All the individuals were non-smokers and were selected after informed oral consent had been obtained.

All the patients (group A) had a history of intermittent chest tightness, wheezing or shortness of breath for at least 30 years prior to study participation consistent with the diagnosis of asthma according to Global Initiative for Asthma. All the asthmatics controlled their condition by using 250 µg to 500 µg of beclomethasone dipropionate daily and short-acting $ß_2$-agonists as needed. All other anti-asthmatic drugs were withdrawn at least 4 weeks prior to the study.

None of the individuals (groups A, B and C) had had any respiratory infections in the month previous to their 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.

Diagnostic Tests

All patients were examined by a physician and underwent spirometric testing using the same equipment (Vitalograph Compact) at least 6 hours after the last dose of any bronchodilator. Predicted values were measured according to Knudson et al [14]. Subjects' spirometric performances were assessed by means of a computerized program following the American Thoracic Society (ATS) 94 criteria. Approval for analysis was determined using the ATS 94 criteria and accuracy was achieved if, within the same evaluation, three curves were acceptable and reproducible.

Twenty to 25 mL of peripheral blood was withdrawn from the vein of the forearm. Peripheral blood cells were stained with monoclonal antibodies anti-CD3 phycoerythrin cyanine 5 (PECy5) (Dako, Denmark), anti-CD4 PECy5 (Dako) anti-CD8 phycoerythrin (PE) (Immunotech, Marseille, France), anti-CD25 fluorescein isothiocyanate (FITC) (Immunotech), anti-TCRγδ phycoerythrin (PE) (Immunotech), anti-HLA-DR FITC (Immunotech), anti-CD95 FITC (Immunotech), used as manufacturer`s specifications. Flow cytometry data were collected on a FACS Calibur device (BD Biosciences, San Jose, California, USA) and analysed using Paint-a-gate (BD Biosciences) software directed at lymphocytes.

Statistical analysis was performed using SPSS 12.0 software package. The Kolmogorov–Smirnov test was used to check if variables were normally distributed. For those with a normal distribution the parametric t test was used for two independent samples. Variables that were not distributed normally were evaluated using the Mann-Whitney nonparametric test. *P* values less than .05 were considered significant.

Statistical comparisons were made between young and elderly control groups (C and B) and between healthy elderly persons (group B) and elderly asthmatics (group A).

Results

All patients (group A) were clinically stable and presented an average percentage of predicted forced expiratory volume in 1 second of 73.6 ± 25.3 mL.

The mean values of CD3, CD4 and CD8 T cells in the blood were within normal ranges, although asthmatic patients showed a tendency to present an increase in $CD4^+$ T cells (Table 1).

The activation marker CD25 was increased in the elderly healthy population (group B) when compared with the adult healthy population (group C). Asthmatic patients (group A) had a decreased expression of CD25 when compared with control elderly subjects (group B) (*P* = .0001). The rise in CD25 expression involved T cells ($CD3^+$). The HLADR values were similar in the three groups studied although asthmatics presented the higher values (Table 2). A statistically significant negative correlation between CD25 and HLADR was found within asthmatics (Pearson correlation coefficient –0.27; *P* = .031).

The apoptosis marker, CD95 was significantly increased in the elderly control group (group B) when compared with the adult control group (group C). Asthmatic patients (group A) showed slightly lower CD95 values than the elderly control group (group B). A statistically significant negative correlation between CD95 and $CD3^+CD25^+$ was found within asthmatics (Pearson correlation –0.26; *P* = .044) but was absent (*P* = ns) in healthy control individuals.

The TCRγδ cells were over 4% both in adults and the elderly control groups and were significantly reduced in asthmatics (Table 3). A statistically significant negative correlation between γδ lymphocytes and CD95 was obtained only within the control elderly group (Pearson correlation –0.44; *P* = .043).

Table 1. Levels of CD3, CD4 and CD8 T Cells in Asthmatics and in the Control Groups

Population	CD3 Mean (±SD)	CD4 Mean (±SD)	CD8 Mean (±SD)
Control - adults (group C)	76.7 ± 4.2	44.8 ± 3.8	25.6 ± 4.5
Control - elderly (group B)	74.8 ± 8.8	43.5 ± 10.2	25.7 ± 10.2
Asthmatics (group A)	74.9 ± 7	48.8 ± 8.7	23.3 ± 7.9

Table 2. Levels of CD25, CD25/CD3 and HLADR in Asthmatics and in the Control Groups

Population	CD25 Mean (±SD)	$CD25^+CD3^+$ Mean (±SD)	HLADR Mean (±SD)
Control - adults (group C)	5.5 ± 2.4	3.6 ± 1.4	15.4 ± 5.1
Control - elderly (group B)	22.4 ± 7.8*	19.3 ± 7.0*	17.8 ± 5.9
Asthmatics (group A)	14.3 ± 5.9	12.3 ± 6.3	18.4 ± 9.2

* Student *t* test, $P < .001$

Table 3. Levels of CD95 and TCRγδ Expression in Asthmatics and in the Control Groups

Population	CD95 Mean (±SD)	γδ Mean (±SD)
Control - adults (group C)	13.8 ± 10.8	4.6 ± 2.1
Control - elderly (group B)	52.6 ± 12.1*	4.1 ± 3.3
Asthmatics (group A)	49.3 ±13.7	2.8 ± 2.1†

* Student *t* test, $P < .001$
† Student *t* test, $P < .05$

Discussion

This study was carried out to evaluate T cell input, mainly the TCRγδ lymphocyte subpopulation and the expression of CD95 in lymphocytes in long-lasting asthma, given the bronchial chronic inflammation that characterizes this disease.

In the elderly there is an underlying chronic inflammatory state with high circulatory levels of pro-inflammatory cytokines and their receptors, so it was decided to include in this study a control adult population to obtain information related to the aging process. According to some studies, in elderly, the pattern of cytokine production is altered with a shift towards a T_H2 response [15,16].

In the present study we found a higher number of $CD4^+$ cells in the asthmatic patients, and these are often correlated with asthma pathogenesis and disease severity [17]. The CD25 values were significantly higher in the elderly control group when compared with the adult cohort. The immune system undergoes considerable qualitative and quantitative changes during aging including the display of a number of activation markers [18,19].

In contrast, CD25 expression was significantly reduced while HLADR was higher in the asthmatic patients when compared with the same age control group. T lymphocytes ($CD3^+CD25^+$) from these patients failed to show an additional rise of this activation marker (when compared with the elderly control group) as has been described in younger asthmatic patients. A statistically significant negative correlation between CD25 and HLADR was established within the asthmatics. This could be associated with the T_H2 cytokine profile environment which is characteristic of asthma and aging and can selectively downregulate IL-2 production and IL-2R expression [3,20].

In this study the elderly population showed a statistically significant increase in CD95 expression when compared with the young control group. The elevated levels of CD95 and a higher sensitivity to apoptosis induction seem to be a general feature of all T cells in the elderly. Apoptosis is involved in many changes which are characteristics of immunosenescence [21]. Although asthmatic patients showed a lower expression of the apoptosis marker CD95, no significant differences were observed between affected and healthy elderly individuals. The patients studied were taking low doses of inhaled steroids and were asymptomatic. According to some studies, steroids can induce apoptosis in cells of asthmatic subjects and can also reduced Fas (CD95) and CD25 expression [1,22]. In addition, the Fas system is less active in the T_H2 environment which is typical of asthma [23-25] A statistically significant negative correlation between CD95 and $CD3^+CD25^+$ was observed within the asthmatics but not in the control group. As the asthmatic patients analysed in the present study were older than 65 and were asymptomatic, the decrease in CD95 observed was not significant. In spite of this, T cells expressing activated markers probably have a lower propensity to apoptosis in asthma even in the elderly.

Although most of the cells participating in the immune defence are αß types, there is data suggesting that in asthma and in rhinitis there is a small subset of pulmonary γδ T cells that produce T_H2-type cytokines and might control the T_H2-driven allergic inflammation [26,27]. These cells have the skill of recognizing different allergens from the environment [28]. In this study the γδ lymphocytes were significantly reduced in long-lasting asthma when compared with the elderly control group. This T cell subset is also involved in the defence against pathogens and can decrease during the ageing process [29]. However, the results of γδ lymphocytes in the healthy adults and the healthy elderly groups were similar, reinforcing the hypotheses that the observed reduction was associated with asthma pathogenesis. A statistically significant negative correlation between γδ lymphocytes and CD95 was obtained only within the elderly control group. In addition CD95γδ percentage values (1.9 ± 1.4), in this healthy population are low (unpublished data) confirming the absence of increased expression of Fas (CD95) in this lymphocyte subset. Despite basal inflammatory changes present

in elderly, the γδ lymphocytes showed no particular tendency to initiate apoptosis [8,30].

Both proinflammatory and antiinflammatory properties have been recognized in TCRγδ lymphocytes [7,31-34]. This T cell subset may develop regulatory activity on allergic inflammation and suppressive effects on airways hyperactivity [35]. This T cell subset can produce high levels of IFN-γ, which reduces IgE production. The reduced number of TCRγδ lymphocytes observed in the patients studied, who presented moderate persistent asthma for more than 30 years, suggests a negligible regulatory activity of these cells on disease evolution. Many studies have been performed in TCRγδ lymphocytes, but some doubts remain about their exact mechanism of action. Their role in inflammation seems to depend on the environmental conditions.

In conclusion, in the elderly there is a higher level of CD95 and CD25 T cell expression. In elderly asymptomatic asthmatics, taking inhaled steroids, these receptors stay within normal range values when compared with the same age group, but T cells displaying activated markers probably have a lower propensity to apoptosis in asthma even in the elderly. γδ T cells may be reduced in long-lasting asthma and may play a minor role in the inflammatory changes of this disease. Finally, long-lasting asthma evaluation should take into account the immunological and inflammatory changes found in the elderly to avoid results being misinterpreted.

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