CONTRIBUTION OF CD4+ T CELLS TO BRONCHIAL WALL INFLAMMATION IN ASTHMA

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CONTRIBUTION OF CD4+ T CELLS TO BRONCHIAL WALL INFLAMMATION IN ASTHMA (Abstract): **Aim:** Asthma is a chronic respiratory condition characterized by a complex interplay between different types of immune cells and morphological structures of the bronchial wall, which contributes to bronchial hyper-reactivity, mucus hypersecretion, and airway narrowing. Within the heterogeneous immunological profile of asthma, CD4+ T cells play an essential role in disease progression, bronchial wall remodeling, and response to treatment. The aim of this study was to evaluate the relationship between CD4+ T cells infiltration in bronchial wall and spirometry parameters in a group of asthmatic patients. **Materials and methods:** This study comprised 21 patients with a history of bronchial asthma, with different degrees of severity based on the latest Global Initiative for Asthma (GINA) classification. All patients underwent a fibro bronchoscopy examination accompanied by tissue biopsy for microscopical analysis. **Results:** The study comprised 21 asthmatic patients, with a female predominance (52.38%) and a mean patient age of 44.1±16.1 years. Microscopic examination revealed a progressive accumulation of inflammatory cells and CD4+ T cells within the bronchial wall, consistent with asthma severity, compared to healthy individuals. The statistical analysis showed an inverse correlation between FEV1 (forced expiratory volume in 1 second) (%) values and CD4 immunoexpression ($r = -0.89$, $p < 0.001$). **Conclusions:** The gradual accumulation of CD4+ T cells in bronchial wall is one the most significant pathological feature, which is predominantly observed in severe asthma and is associated with impaired lung function. **Keywords:** ASTHMA, CHRONIC INFLAMMATION, CD4+ T cells, FEV1.

INTRODUCTION

Asthma is a chronic disease that affects over 300 million people worldwide (1). Its major pathological feature is the persistent inflammation of the bronchial wall, which leads to episodes of wheezing, mucus hypersecretion, and coughing. This chronic bronchial wall characteristic is supported by the persistent inhalation of allergens and irritants, which may lead to airway remodeling (2). This process is characterized by subepithelial fibrosis, goblet cells hyperplasia, glandular and smooth muscle hypertrophy, and neoangiogenesis in lamina propria (3, 4).

Chronic inflammatory processes in the bronchial mucosa may sometimes persist even in the absence of symptoms (1). This feature supports the importance of long-term anti-inflammatory treatment, in which

corticosteroid therapy remains the standard option (2).

The diagnostic tools, such as fractional exhaled nitric oxide (FeNO), spirometry exam, periostin serum level, blood eosinophil count, and sputum analysis, facilitate the quantification of airway inflammation and guide the asthma therapy (5). However, in recent decades, numerous studies have focused on the evaluation of CD4+ T cell population and the profile of cytokines they produce, given that the most of the asthmatic patients exhibit a type 2 inflammatory pattern (6, 7).

Despite significant progress in understanding the immunological mechanisms in asthma, the heterogeneity of immune cell profiles observed in asthma remains only partially understood. In this context, our study aims to explore the relationship between the inflammatory profile and spirometry parameters in a group of asthmatic patients, focusing on the assessment of CD4+ T cells infiltration of the bronchial wall.

MATERIALS AND METHODS

The study group comprised 21 asthma patients under inhaled corticosteroid therapy, diagnosed in the Specialty Ambulatory of the Pneumology Clinic Hospital, Iasi, Romania. All patients were over 18 years old, were non-smokers (either never-smokers or former smokers), and had no other acute or chronic pulmonary pathology at the time the pulmonary function tests were performed. All patients underwent a spirometry exam and were classified according the last GINA classification (8).

Fragments of bronchial mucosa were collected by fibro bronchoscopy for microscopic examination. To avoid insufficient sample collection or inconclusive results, five to seven bronchial tissue samples were obtained for each patient by fibro bronchoscopy exam (4). The bronchial tissue samples were processed by the standard paraffin embedding method and stained with Hematoxylin & Eosin (H&E staining). To highlight the presence of T4 lymphocytes in the bronchial mucosae, an immunohistochemical exam was performed using anti-CD4 antibodies (clone 4B12, M7310, Dako), following the manufacturer's protocol. Semi-quantitative assessment of CD4 immunoexpression was conducted for each case by examining five representative positive areas at 40× magnification, according to the following scoring system (9, 10): no CD4 positive cells (score 0), <10% CD4 positive cells (score 1), 10-50% CD4 positive cells (score 2), and >50% positive cells (score 3). The control group comprised two asymptomatic, non-asthma patients evaluated for suspected tumors.

The statistical analysis was conducted using SPSS version 25 (IBM, Armonk, NY, USA). Correlations were evaluated using Pearson's and Spearman's coefficients, and linear regression analysis was applied to estimate the slope of the relationships. A p value < 0.05 was considered statistically significant.

All procedures complied with international research ethics standards, including those outlined in the Declaration of Helsinki for research involving human subjects and patients.

RESULTS

The study group consists of 11 women (52.38%) and 10 man (47.62%), with a median age of 44.1±16.1 years, most of them living in urban areas (n = 17; 80.95%). The median duration of the disease was 16.6±9.7 years and the mean

FEV1 value (% of predicted) was 79.3±20.3%. Following the application of GINA criteria, the patients were classified into three degrees of asthma severity: 10 cases (47.62%) of well-controlled (intermittent/mild) asthma, eight cases (38.10%) of partially controlled (moderate) asthma, and three cases (14.28%) of uncontrolled (severe) asthma.

A progressively increased amount of immune cells infiltration in lamina propria has been detected in all asthmatic cases compared with control cases. The immune infiltrate had a mixed composition, predominantly including lymphocytes, along with some eosinophils and neutrophils, the latter being especially detected in severe forms of asthma (figs. 1 and 2).

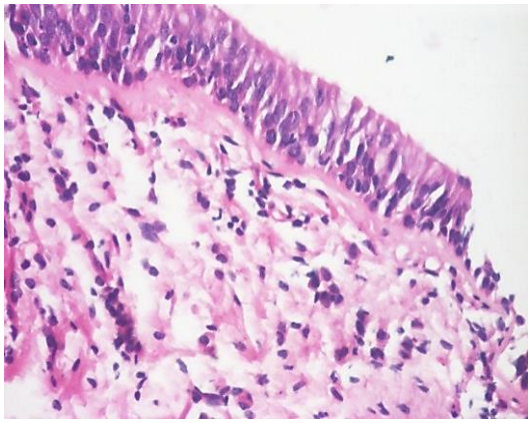

Fig. 1. Moderately abundant immune infiltrate in lamina propria in partially controlled asthma, H&E staining, 20x

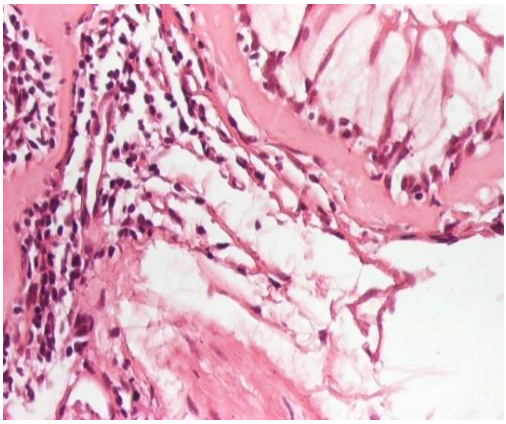

Fig. 2. Intense mixed inflammatory infiltrate in the lamina propria in uncontrolled asthma, H&E staining, 20x

Regarding CD4 expression, rare CD4+ T cells have been observed in control cases (fig. 3). A mildly abundant CD4+ lymphocytic infiltrate (score 1) was observed in the superficial lamina propria of the bronchial mucosa in all well-controlled asthma patients (fig. 4), while this infiltrate was also detected in deep lamina propria (score 2) in partially controlled cases. Additionally, a diffuse CD4+ infiltrate involving the entire bronchial mucosa was observed in all three cases of uncontrolled asthma and in two partially controlled asthma patients (score 3) (figs. 5 and 6) (tab. I).

TABLE I.

CD4 immunoexpression distribution in the study group

CD4+ score	Well-controlled Asthma (n; %)	Partially controlled asthma (n; %)	Uncontrolled Asthma (n; %)
Score 1	9 (42.86%)	0	0
Score 2	1 (4.76%)	6 (28.58%)	0
Score 3	0	2 (9.52%)	3 (14.28%)
Total	10 (47.62%)	8 (38.10%)	3 (14.28%)

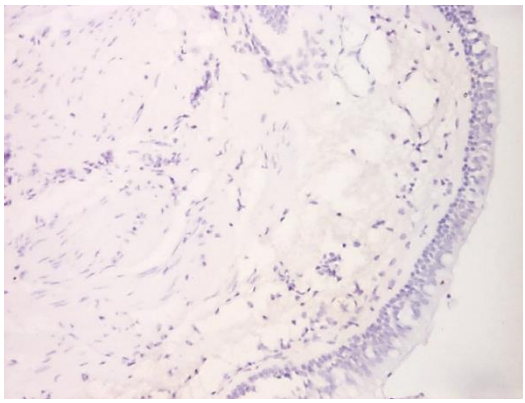

Fig.3. Rare CD4+ T cells in lamina propria in a control case, 4x

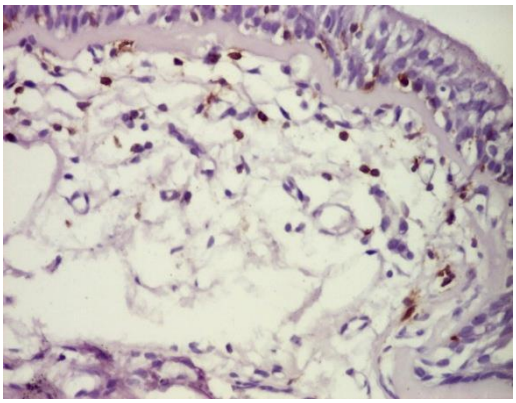

Fig.4. CD4+ mild lymphocytic infiltrate in lamina propria in well-controlled asthma, 20x

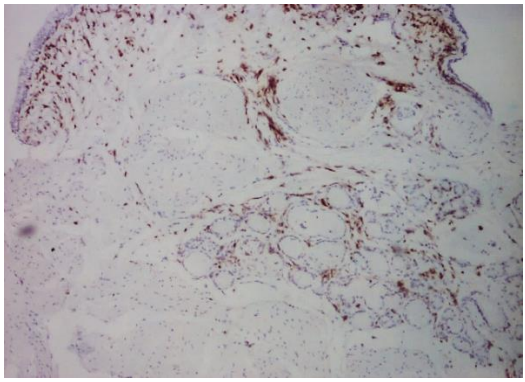

Fig. 5. Diffuse CD4+ lymphocytic infiltrate involving the deep part of the bronchial wall in uncontrolled asthma, 4x

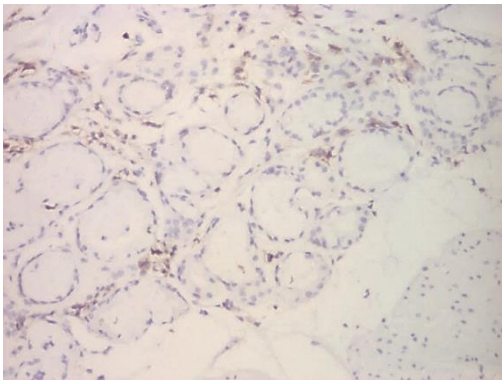

Fig. 6. CD4+ T cells infiltrating the glandular area of the bronchial wall in uncontrolled asthma, 20x

Statistical evaluation showed a significant association between CD4 immunoexpression and asthma severity (p = 0.00016) (tab. II).

TABLE II.

Statistical parameters for the association between CD4 immunoexpression and bronchial asthma severity

	Pearson's chi-square test	df	p 95% CI
Pearson's chi-square - χ^2	26.75556	df=6	0.00016
M-L Chi-square	30.57868	df=6	0.00003
Correlation coefficient (Spearman Rank R)	0.8959092		0.00000

CI - confidence interval; df - degrees of freedom; M-L Chi-square - Maximum Likelihood Chi-Square

Additionally, FEV1 values were significantly lower in cases with a high CD4 score. Thus, a mean FEV1 value of 98.4±8.2 % was recorded in asthma cases that exhibited a weak CD4 positive immunoexpression and a mean FEV1 value of 54.1±7.4 % was registered in asthmatic patients that had high CD4 positive immunoexpression (tab. III). This inverse association between CD4 immunoexpression score and FEV1 values (%) was confirmed by the high correlation coefficient (r = -0.89, $p < 0.001$), and was reflected by the negative regression slope (fig. 7).

TABLE III.

FEV1 statistical indicators according to CD4 immunoexpression

CD4	FEV1 (%)		
	Mean ±SD	Minimum; Maximum	Median
Score 1	98.4% ±8.2	88.0; 116.0	96.3
Score 2	72.8±11.2	62.0; 93.5	69.2
Score 3	54.1±7.4	45.0; 62.0	53.7

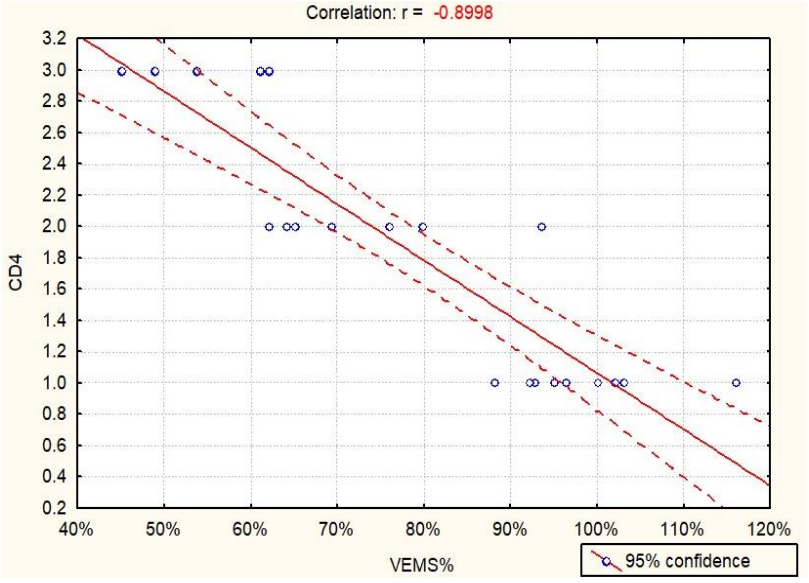

Fig. 7. The regression line showing a negative correlation FEV1 *vs.* CD4 immunoexpression

DISCUSSION

In an analogous manner to other chronic inflammatory diseases, asthma poses significant challenges for both diagnosis and management (1, 11 - 13).

Asthma encompasses a complex of pathological features associated with bronchial wall remodeling, due to a T cell-driven chronic inflammatory process (14). CD4+ T cells, particularly Th2 cells, release a panel of pro-inflammatory cytokines, such as IL-4, IL-5, and IL-13, which support the mucus hypersecretion, eosinophils recruitment and airway hyper-

responsiveness in asthmatic patients (14, 15). Consequently, suppressing chronic airway inflammation with corticosteroids is still the standard treatment in patients with asthma, as these drugs reduce type 2 cytokine–producing $CD4^+$ T cells, which act as key drivers of the recruitment and activation of innate immune cells (e.g., mast cells and eosinophils) within the bronchial wall (16).

The conventional approach involves the stratification of asthma into three distinct endotypes: type-2 high, type-2 low, and mixed endotypes (17, 18). Based on this classification, different studies have shown that Th2, Th9, follicular helper T (Tfh), and tissue-resident memory T (TRM) CD4+ T cell subtypes are linked to the development of eosinophilic asthma (type-2 high asthma), while Th1 and Th17 CD4+ T cell subtypes modulate the development of neutrophilic asthma (type-2 low asthma) (2, 16). Additionally, a recent study suggested that the increased senescent $CD4^+$ T (Tsen) cells in asthmatic patients is likely mediated by type 2 inflammation, compared with healthy controls, and it correlates with asthma severity and elevated FeNO values (19). Thus, potential new therapeutic approaches for asthma management could be related to a deeper understanding of the molecular mechanisms involved in CD4+ T cells and their subsets contribution to the airways remodeling.

In this context, our study supports the relationship between asthma severity and the degree of CD4+ T cell infiltration of the bronchial wall (p <0.001), a CD4 higher score being associated with a lower FEV1 value. Moreover, in partially controlled and uncontrolled asthma cases, the CD4+ T cells deeply infiltrate the bronchial wall, affecting its glandular areas. Our results are consistent with other previous studies, which explored the cellular inflammatory profile in asthmatic patients (20 - 22). For example, a consistent CD4+ T cells infiltrate has been detected in a group of patients with moderate-to-severe persistent asthma, compared to controls or patients with sarcoidosis (20). Another study has shown that $CD4^+$ T cell counts in bronchial mucosae may predict subsequent decline of the lung function in patients with mild to moderate forms of asthma ($p = 0.04$ pre-bronchodilator and $p = 0.03$ post-bronchodilator) (21). Furthermore, an increased serum level of IL-2 ($p < 0.001$) was registered in acute asthma, which indirectly reflect the activation of CD4+ cells in the bronchial wall (22). Eosinophilia and CD4+ T cells activation level have been also correlated with the coefficient of variation of peak expiratory flow in asthma cases, suggesting their potential role in the airway inflammation evaluation (23). Last but not least, a high proportion of $CD4^+$ TRM (tissue-resident memory T) cells, comprising nearly 65% of all $CD4^+$ T cells, was identified in the bronchial wall of severe asthma patients, whereas mild (well-controlled) asthma patients showed only 13% $CD4^+$ TRM cells (16). Their activation supports the release of pro-inflammatory non-Th2 cytokines, such as TNF, CCL3, and CCL5, which contribute to the bronchial wall chronic inflammatory profile in asthma (16).

Taking together, these data support CD4+ T cells role in bronchial wall remodelling, while the intensity of the chronic inflammatory infiltrate is associated with the severity of the patients' clinical symptoms.

Currently, the therapeutic management

in asthma has considerably improved lung function and quality of life in asthmatic patients, by adding in the therapeutic scheme of various anti-inflammatory drugs, such as anti-IL-5, anti-IL-4/IL-13 or anti-IgE therapies, to standard inhaled corticosteroid treatment (1). Despite this therapeutic progress, currently available biological drugs are mostly ineffective in patients with type-2 low or steroid-resistant asthma. Therefore, further research targeting the immunological profile of asthma is required to develop personalized therapies

CONCLUSIONS

Asthma is a chronic inflammatory disorder of the bronchial wall, $CD4^+$ T cells playing a key role in the orchestration of the immune response by driving eosinophilic inflammation, mucus hypersecretion, and airway hyper-responsiveness.

A higher accumulation of CD4+T cells in the airway is correlated with lower FEV1 and faster decline of the lung function. Elucidating the functional roles of $CD4^+$T cells is critical for the designing effective therapeutic strategies in asthma and the predicting patient prognosis. The future of asthma treatment lies in precision immunotherapy and personalizing therapy to target the unique immune profile of each patient.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this research.

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